THE JOURNAL OF DAIRY RESEARCH

EDITED FOR

The Committee of Management

BY

A. T. R. MATTICK, B.Sc., Ph.D.
The National Institute for Research in Dairying, University of Reading

and

N. C. WRIGHT, M.A., D.Sc., Ph.D.
The Hannah Dairy Research Institute, Kirkhill, Ayr

ASSISTED IN THE SELECTION OF PAPERS FOR PUBLICATION BY

Dr L. BULL (Australia)
Prof. E. L. CROSSLEY (Reading)
Prof. D. B. CUTHBERTSON (Aberdeen)
Prof. I. de BURGH DALY, F.R.S. (Cambridge)
Dr H. P. DONALD (Edinburgh)
Dr PAUL FILDES, F.R.S. (London)
Prof. P. J. J. FOURIE (South Africa)
Prof. R. C. GARRY, F.R.S.E. (Glasgow)
Dr T. GIBSON (Edinburgh)
Dr J. HAMMOND, F.R.S. (Cambridge)
Dr J. O. IRWIN (London)
Dr A. G. LOCHHEAD, F.R.S. (C.) (Canada)
Prof. F. C. MINETT (Pakistan)
Prof. W. RIDDET (New Zealand)
Dr K. C. SEN (India)
Sir JAMES A. SCOTT WATSON (London)

VOLUME 17

CAMBRIDGE
AT THE UNIVERSITY PRESS
1950

Committee of Management

PROF. H. D. KAY, C.B.E., F.R.S. (*Chairman*)
PROF. R. C. GARRY, F.R.S.E.
MR W. H. SENIOR
DR J. A. B. SMITH
DR E. MARSDEN
SIR HERBERT HOWARD
DR A. T. R. MATTICK, DR N. C. WRIGHT } *Editors*
MR H. F. BURGESS (*Secretary*)

Librarian and Editorial Assistant

MISS D. KNIGHT, B.A., F.L.A. Dip. Lib.

Printed in Great Britain at the University Press, Cambridge
(Brooke Crutchley, University Printer)
and published by the Cambridge University Press
London: Bentley House, N.W.1
American Branch: New York
Agents for Canada, India and Pakistan: Macmillan

CONTENTS

No. 1 (February 1950)

Original Articles: PAGE

406. The sale of veal calves and veal production in West Wales (1943–6). R. Phillips 1

407. The number of sweat glands in the helix of the cow's ear and the milk yield. J. D. Findlay, A. Myfanwy Goodall and S. H. Yang . . 22

408. Udder infections in the 'dry period'. I. F. K. Neave, F. H. Dodd and Ettie Henriques 37

409. Apparatus for studies on disinfection of milking machine teat cups. G. E. K. Lineham 50

410. The oxidation of ascorbic acid in dairy products. J. E. Allan . . 54

411. *Pseudomonas putrefaciens* in New Zealand butter. G. I. Thomson . 66

412. Coliform bacteria in New Zealand butter. G. I. Thomson . . . 72

413. The growth requirements of lactobacilli in relation to cheese flavour development. G. J. E. Hunter 79

Reviews of the Progress of Dairy Science:

Section F. Milk-borne diseases. J. Smith 91

No. 2 (June 1950)

Original Articles:

414. Experiments on milking technique. 7. The effect of subjecting dairy cows, for a complete lactation, to a rigid control of the duration of milking. F. H. Dodd, A. S. Foot, Etty Henriques and F. K. Neave 107

415. Factors affecting the rate of machine milking. Eleanor S. Baxter, Pamela M. Clarke, F. H. Dodd and A. S. Foot 117

416. The effect of bacterial infection on the milk yield of the individual quarters of the cow's udder. Joan V. Crossman, F. H. Dodd, J. M. Lee and F. K. Neave 128

417. Keeping quality and raw-milk grading. I. Dye tests as measures of the keeping quality of milk. A. Rowlands, H. Barkworth, Zena Hosking and O. Kempthorne 159

418. Device for preparing roll-tube cultures. G. M. Moir and G. I. Thomson 192

419. The phosphatase test of Aschaffenburg and Mullen. Use of permanent colour standards and comparison with the Kay-Graham test. J. Tramer and J. Wright 194

420. The colloidal phosphate of milk. I. Composition and titrimetric estimation. G. T. Pyne and J. J. Ryan 200

421. Haemolytic streptococci in cheese. C. L. Hannay and L. G. Newland 206

422. Cheese discoloration: oxidation of bixin in annatto-coloured cheeses promoted by sulphydryl compounds. C. R. Barnicoat 209

No. 3 (October 1950)

Original Articles: PAGE

423. Some observations on the behaviour of dairy cattle with particular reference to grazing. M. E. Castle, A. S. Foot, and R. J. Halley . 215

424. A study of lactation as affected by heredity and environment. L. C. Sikka 231

425. Further investigations on the presence in raw milk of a bactericidal substance specific for certain strains of coliform organisms. C. S. Morris and M. A. Edwards 253

426. Some bacteriological aspects of the deterioration of pasteurized milk. T. R. Ashton 261

427. The acid phosphatase of cows' milk. I. Some properties of the enzyme. J. E. C. Mullen 288

428. The acid phosphatase of mammary tissue of the cow and the rat. J. E. C. Mullen 306

429. Studies on Egyptian Domiati cheese. A. H. Fahmi and H. A. Sharara 312

430. Studies of the nature of subjective judgements in cheese-grading. R. Harper and M. Baron 329

431. Two samples of 7-year-old Cheddar cheese. Edith R. Hiscox . . 336

Reviews of the Progress of Dairy Science:

Section A. Physiology of dairy cattle. I. Reproduction and lactation. J. A. B. Smith 338

SEP 11 '52 BINDERY

Jnl. o

88011

THE JOURNAL OF THE

SOCIETY OF DAIRY TECHNOLOGY

17 DEVONSHIRE STREET, LONDON, W.1. *Tel. Langham* 5059

The Journal is issued in quarterly parts, four parts constituting a volume. The parts are usually issued in January, April, July and October each year.

The Subscription rate to non-members of the Society, payable in advance, is £2 net per volume (post free): single numbers, 12*s.* 6*d.* net. Subscriptions and all business enquiries should be sent to the Secretary, Society of Dairy Technology, 17 Devonshire Street, London, W.1.

Each member of the Society receives one free copy of each number. One further copy of each number may (if available) be bought by members for 7*s.* 6*d.*

CONTENTS OF THE APRIL ISSUE

The Past One Hundred Years, by E. CAPSTICK. The Road Ahead in the Milk Industry, by BEN HINDS, J.P. Types of Organisms Present in Commercially Pasteurized Milk, by J. W. EGDELL and E. R. BIRD. The Design and Operation of the Cheese Trommel—Its use in Cheddar Cheese Making, by J. M. SHARKEY. Change of Temperature of Milk in Transit from the Farm to the Creamery, by I. JENKINS, E. M. REEVE and A. L. PROVAN. Heat Resistant Bacteria in Raw Milk: Part I, by S. B. THOMAS, DOROTHY ELLISON, D. G. GRIFFITHS, E. JENKINS and K. J. MORGAN. Part II, by S. B. THOMAS, DOROTHY ELLISON, D. G. GRIFFITHS, M. HUMPHREYS, W. L. R. VAUGHAN, G. GEORGE and E. P. DAVIES. The Dairyman's Approach to Safe Milk, by B. M. FISCHER. Electricity and Milk Production, by C. A. CAMERON BROWN. Sectional Notes.

The Journal publishes not only papers read at meetings of the Society, but also papers on dairy technology by members and non-members.

Advertising Offices: 24 BRIDE LANE · FLEET STREET · E.C.4

Indian Journal of Dairy Science

Established in 1948 as a medium for the advancement and dissemination of knowledge in regard to dairy science. Subscription Rs 12 per Volume of 4 parts, payable in advance. Single copies Rs 3 each. Subscriptions may be sent to the Hon. Joint Secretaries, Indian Dairy Science Association, Hosur Road, Bangalore 1, India.

Chief Editor: K. C. SEN, D.SC., F.N.I.

CONTENTS OF VOL. III, No. 1, MARCH 1950

V. R. Bhalerao and K. P. Basu: Effect of different milk supplements on the poor South Indian diet.

T. M. Paul and C. P. Anantakrishnan: Colostral fat of goats and sheep.

J. V. Bhat and K. Sethna: Microflora of ghee.

N. M. Patel, B. M. Patel and R. M. Patel: The influence of different levels of cutting and stage of growth on the forage value of some well-known grasses.

S. C. Ray and A. Mitra: Design of tamper proof devices in distribution cans to prevent adulteration of milk.

S. N. Anantaramiah, C. P. Anantakrishnan and K. C. Sen: Studies on vitamin A in milk. Part I. Effect of varying carotene intake on the vitamin A potency of buffalo milk and butter fat.

SEP 11 52 BINDERY

636.2.088.31

406. THE SALE OF VEAL CALVES AND VEAL PRODUCTION IN WEST WALES (1943–6)

BY R. PHILLIPS
University College of Wales, Aberystwyth

(With 10 Figures and 6 Maps)

In years gone by, the three counties constituting the west Wales region (Cardiganshire, Carmarthenshire and Pembrokeshire) were devoted to mixed farming concentrating mainly on the rearing of cattle, sheep, horses and pigs, together with some fattening for meat. The rapid expansion of dairy farming in the region, particularly during the war years, resulted in a corresponding decline in lowland sheep farming and in cattle rearing.

The major factors contributing to the decline were the establishment in 1933 of the Milk Marketing Board (M.M.B.); the war-time priority propaganda for milk and changes in the relative prices of meat and milk. Carmarthenshire, it is true, had already become 'milk-minded' some time beforehand as Davies describes(1), but it was not until the above factors became truly operative that the change in the system assumed significance.

The west Wales region consists of approximately 1¼ million acres, one-third of the whole of Wales and Monmouthshire, of which 70% is devoted to crops and grass and 30% to rough grazing. It supports a population of nearly 300,000 cattle (nearly one-third of that of the whole of Wales), of which 40–45% are cows, and in 1939 it carried a population of nearly 850,000 sheep (4 June), which by 1947 had dwindled to nearly a half.

One of the most important results of the change-over to milk selling has been a steady increase in the sale of 'surplus' calves, and since 1940 this has been done weekly or fortnightly at the twenty-five fat-stock collecting centres of the Ministry of Food, which were established in that year. These calves, males for the most part, were bought by the Ministry of Food in the categories 'bobbies' (calves a few days old), 'veals' (a few weeks old) and 'casualties'. The latter are dealt with later in the paper, but it should be pointed out at this stage that the percentage of incidence is so low that it is scarcely a significant factor and does not materially affect the statistics recorded. Table 1, showing prices, illustrates the relative value of the different categories.

Table 1

Calves (per lb. estimated dressed carcass weight)

First quality veal	13*d.*
Second quality veal	11*d.*
Third quality veal	9*d.*
'Stirky' calves for manufacturing purposes	6½*d.*

Bobby (slink) calves suitable only for manufacture (per head)

Large	30*s.* (valued at 9*d.* per lb.)
Medium	25*s.*
Small	18*s.*
Very small	10*s.*
Unfit for human food	2*s.* 6*d.*

Fortunately, the chairman of auctioneers (Major Francis, Carmarthen), who is responsible for operating the Fat-Stock Scheme for the Ministry, has kept a *monthly* record of the sales of calves at the marts of each centre since its inauguration. A paper dealing with

Map. 1. Collecting areas, west Wales (Cardiganshire, Carmarthenshire, Pembrokeshire). County boundaries, ····. Numbers in brackets: (1) weekly marts; (2) fortnightly marts.

Map 1A. Sales of veal calves (and total calf sales in brackets) in thousands. Statistics on which the discussion is based, 1943–6. For the following centres statistics are based on years 1944–6: St Clears, Kidwelly, Llandilo, Llangadock, Llandovery.

the seasonality of the calf sales has already been published (Phillips & Davies(2)). Since 1943, the full records of each weekly or fortnightly mart have also been kept (very nearly

complete), and in these the numbers and weights of the veal calves and the number and grade of the bobby calves are available.

For purposes of convenience the west Wales area is being considered broadly as the area occupied by the administrative counties Cardiganshire, Carmarthenshire and Pembrokeshire, but it should be pointed out that although the boundaries between the three areas to which we shall refer by those names are not strictly the administrative boundaries, the differences balance one another and have no material bearing on the following discussion. Within those counties are the twenty-five marts or collecting centres, to each of which has been allocated a convenient number of surrounding parishes from which it draws its supplies of calves and fat stock. Map 1 gives the name of each collecting centre, the outlines of the collecting parishes and in brackets the figure (1) or (2) which denotes the weekly or fortnightly mart; Map 1A gives the statistics of the sale of veal calves, and in brackets the figures of the corresponding sales of all calves through each collecting centre during the 4 years 1943–6.

This paper is based on an analysis of the mart records of the collecting centres for the 4 years 1943–6, with particular reference to veal production—one of the important results of the recent developments in dairying. It is proposed to deal with veal production from the point of view of (*a*) the west Wales region as a whole; (*b*) the individual counties; (*c*) the individual collecting centres.

The region itself is remarkably self-contained as far as livestock and particularly fat-stock sales are concerned, since the area is bounded by the sea on three sides, and by a mountainous hinterland on the fourth, which means that there are very few road exits to collecting centres situated immediately beyond the boundary. Consequently, the sales through all the collecting centres represent very largely the production within the region, and particularly is this true in the case of surplus calves. In the same way, the sales through the twenty-five collecting centres represent the output of each surrounding area, and it is obvious from Maps 1 and 1A that the collecting areas differ in size and in the volume of the calf trade. It is also to be noted that for the convenience of farmers, collecting centres occasionally accept stock from areas other than their allocated parishes, but a balance of this traffic is maintained since the interchange appears to be mutual in all centres.

The twenty-five collecting centres have been allocated to the three counties as follows:

Cardiganshire (6)	Carmarthenshire (9)	Pembrokeshire (10)
Aberystwyth	Llanybyther	Crymmych
Tregaron	Newcastle Emlyn	Clynderwen
Lampeter	Whitland	Mathry Road
Ystrad	St Clears	Croesgoch
Llandyssul	Carmarthen	Clarbeston Road
Cardigan	Kidwelly	Hasguard
	Llandilo	Haverfordwest
	Llangadock	Narberth
	Llandovery	Pembroke
		Kilgetty

1. THE WEST WALES REGION

(*a*) *Veal production in general* (1943–6)

For convenience, it is at first considered advisable to compare the annual sales of all surplus calves from 1940 to 1948 and to give the annual number of veal calves sold during the years 1943–7.

These are shown graphically in Fig. 1*a*, from which it is seen that there has been a continuous increase in the sale of surplus calves from 1940 to 1946 (the peak year), after which there was a drop in 1947 to the 1945 level and a further drop in 1948. The graph for the sale of veals is parallel to that for the total sales. The drop after 1946 may be a reflexion of both the severity of the winter of 1946–7 with its loss of calf life and of a tendency to return to more rearing after the calf subsidy scheme had become operative. Fig. 1*a* also shows the insignificant numbers of calves sold by the Ministry to farmers for further rearing. Details of the annual sales are given in Table 2.

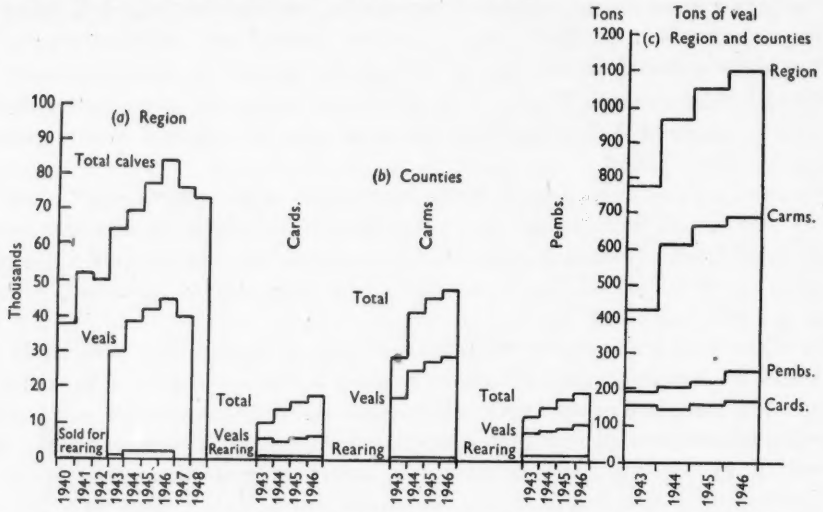


Fig. 1. Annual sales of calves, 1940–8, in west Wales; and of veal calves in the region and the three counties, 1943–7.

Table 2. *Annual numbers of calves and of veal calves sold to the Ministry of Food in west Wales*, 1943–6

Year	No. of calves sold: Total	No. of calves sold: Veal	Percentage of veal calves sold	Carcass wt. of veal: lb.	Carcass wt. of veal: tons	Average carcass wt. of veal calves (lb.)	Sold for rearing: No.	Sold for rearing: Percentage of total calf sales
1943*	50,631	29,909	59·0	1,734,717	774·4	58·0	1,272	2·5
1944	68,804	38,582	56·1	2,172,340	970·0	56·3	2,022	2·9
1945	77,757	42,056	54·1	2,349,372	1,048·8	55·8	1,779	2·3
1946	83,820	45,012	53·7	2,467,796	1,101·7	54·8	1,939	2·3
Total	281,012	155,559	55·0	8,724,225	3,894·6	56·0	7,012	2·5

* The records do not include five centres in Carmarthenshire for 1943.

It is clear from the table that during the 4 years 1943–6 inclusive, there was a gradual increase in the total number of calves sold to the Ministry of Food. But although there was a simultaneous increase in the number of veal calves sold, there was a *decrease* in the *percentage* of veal calves sold, and the inevitable conclusion is that an increasingly large percentage of calves other than veals, i.e. bobbies, for the most part, were being sold to the Ministry. It is interesting to note that the development of dairying in the region as a whole has not only resulted in the sale for slaughter of calves which would previously have been

retained or resold for purposes of rearing, but also in a decline in quality of the calves themselves. Farmers have obviously found it less remunerative to sell milk-fed veals 2–3 weeks old than to sell the calves at birth as bobbies, and thereafter the milk they would have consumed. Table 1 is a guide in estimating the approximate loss in raising a large-sized bobby calf to the average veal weight of 56 lb. A large-sized bobby, worth 30*s*., is valued at the rate of 9*d*. per lb. and it weighs approximately 40 lb. (carcass weight) and 80 lb. (live weight). In order to gain the 32 lb. which will give it a live weight of 112 lb. (equivalent to the average veal carcass weight of 56 lb.) it must be fed for 2–3 weeks on approximately 32 gal. of milk, since it is reckoned that 1 gal. of milk gives an increase of 1 lb. live weight.

The difference between the price of a 40 lb. bobby at 9*d*. per lb. (£1. 10*s*.) and the price of 56 lb. veal at 11*d*. per lb. (£2. 11*s*. 4*d*.) is £1. 1*s*. 4*d*., a poor return for 32 gal. of milk at the contemporary wholesale price, particularly in winter.

It may be argued that veal calves are usually suckled, and that the labour costs of milking should be deducted from wholesale prices, but even then the sale of veal at current prices is not a profitable line of production, and the fact that veal prices have remained stationary for so long indicates lack of encouragement to intensify production. In other words, large-sized veal calves are likely to be produced only by small-holders who do not sell milk, or by milk-selling farmers when they have cows difficult to milk or nervous first-calvers. On a dairy farm, calf-suckling is sometimes resorted to in drying off cows at the end of their lactation; but whatever the reason for veal production there is no doubt that it is less remunerative than milk-selling, and therefore a very high proportion of the surplus calves sold for slaughter are the bobbies.

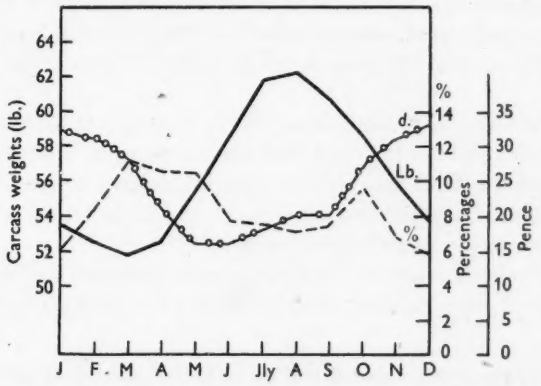


Fig. 2. Seasonality of sales and weights of veal calves in west Wales; 4-year averages, 1943–6. ——, average carcass weight; - - -, percentage distribution. Also wholesale producers' prices for milk (pence per gallon), 1943–6. -o-o-, milk prices.

(*b*) *The variation in veal production from season to season* 1943–6

The varying extent of veal production from season to season must obviously depend ultimately upon the varying number of calvings per season and on the relative price levels of milk and veal. These relationships between 1943 and 1946 are illustrated in Fig. 2 which also shows seasonal variation of veal production from year to year.

Fig. 2 brings out three important features of seasonal veal production in west Wales:

(1) The monthly variation in the percentage of veals sold. The peak percentage occurs in the spring (March) which is the time of the highest calving rate (Phillips & Davies(2)). The secondary peak in October reflects the annual effort to increase the calving rate in autumn.

(2) The monthly average carcass weights of veal calves are lowest in the spring (usually March), while the highest are in July and August, with a difference of 11·1 lb. between the minimum and the maximum weights.

(3) The monthly average wholesale producers' price per gallon of milk (M.M.B., south Wales regional prices) shows lowest milk prices in May and June and highest in December.

It will be noticed that the heavy-weight veal carcasses are produced when milk is relatively cheap, whilst the light-weight carcasses are produced at the time of peak calvings (March) when milk is relatively dear. Clearly, dairy farmers have found it more profitable to sell the largest number of light-weight calves in spring, at the time of maximum calvings and good milk prices, and to sell fewer, heavier calves later on when milk is cheaper. With the annual improvement in milk prices, the average weight steadily declines and the only reason for a late summer increase in numbers is the rise in the autumn calving peak.

The lag between the time of the lowest price of milk (May and June) and the time of maximum average carcass weight in August is due to about 3 months' feeding of potential vealers on low-priced milk.

(*c*) *Annual changes in the monthly production of veal* (1943–6)

It has been shown in Table 2 and Fig. 1 that total calf sales, veal calf sales and the total weight of veal production have increased annually from 1943 to 1946. Fig. 3*f–i* brings out the differences in greater detail, showing monthly production, and it is interesting to note both the steady lowering of the peak figure and the variation of peak month between March and May.

A rather more revolutionary change is evident in successive October peaks. The gradual increase in this figure between 1943 and 1946 has continued in inverse ratio to the spring peaks until the former actually exceeded the latter in this last year (1946). Fig. 3*j–m* shows that veal production is now influenced not so much by the traditional demands of the Easter luxury trade as by normal calving peaks.

The ratio of the sales of vealers to total calf sales is illustrated in Fig. 3*e*, and shows an interesting feature of being regularly irregular during the various months.

(*d*) *Monthly ratios of the sale of veal calves to the total sale of calves* (*Fig.* 3*e*)

It is interesting to attempt to account for the four regular annual peaks of December/January, May, July and October, and the four troughs of March, June, September and November. A comparison of this graph with the bottom section of Fig. 3*j–m* (monthly sales of all calves 1943–6) shows that the March trough in veal calf sales coincides with the highest peak of total calf sales, and incidentally comes towards the end of the winter-feeding period. The July peak in veal calf sales occurs at the period of minimum calvings and minimum or very low total sales, and quantity thus gives way to quality for a time. Only in October do the peaks of both total and veal calf sales occur simultaneously. It is possible that this coincidence, in contrast to the March trough in the sales of vealers and

March peak in the total sales, is a reflexion of the different diets upon which spring and autumn calvers have lived during pregnancy. The superior diet enjoyed by the autumn calvers during the preceding summer may well produce a calf superior to that of the spring calver which has existed on a winter diet during the greater part of pregnancy. Since this

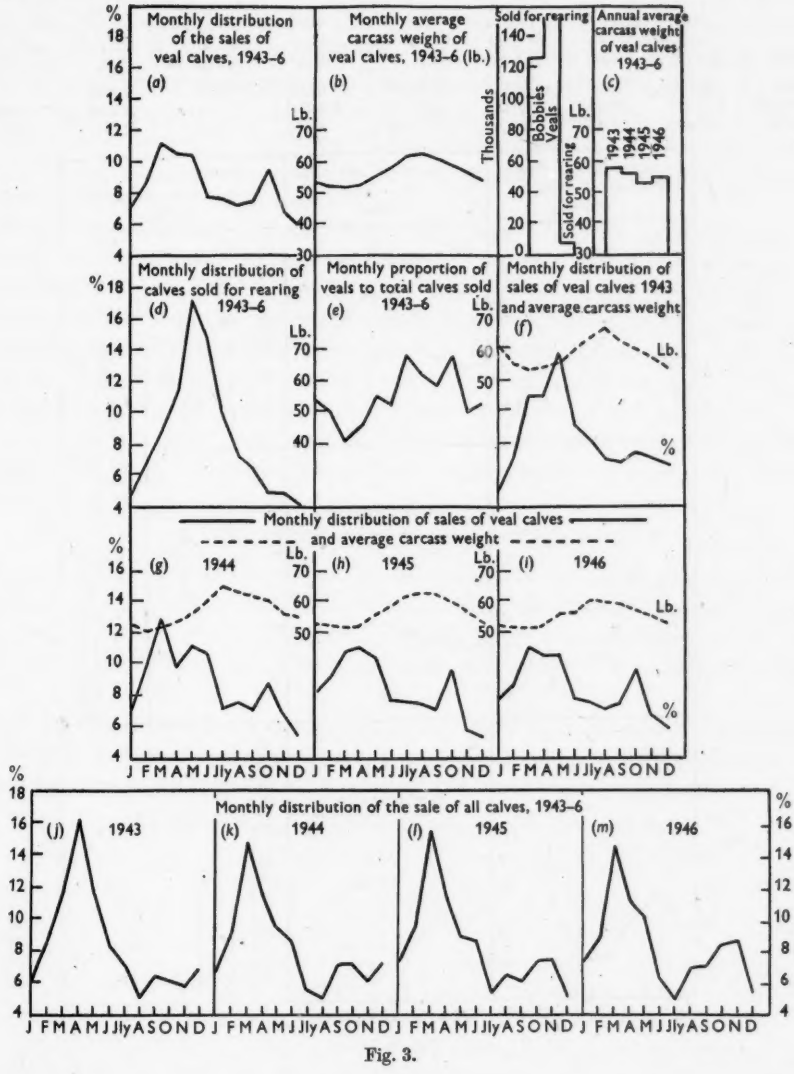


Fig. 3.

has been shown to be true in the case of ewes and lambs (Wallace (4)), there seems to be no reason why it should not apply to cows and calves; at any rate, autumn-born calves are easier to rear and make better live-weight gains than spring-born calves (Phillips (3)), and the fact that on the whole there is a greater proportion of veal calves in the total sales in

summer than in winter does not mean that a greater *number* of veal calves are sold then, for total sales are then at their lowest ebb.

Table 3. *The total number of calves, veal calves, weights and percentages for each of the three counties for the 4-year period* 1943–6

County	No. of calves		Percentage of veal calves	Carcass wt. of veal		Average carcass wt. of veal calves	Sold for rearing	
	Total	Veal		lb.	tons		No.	Percentage of total calf sales
Cardigan	55,684	22,837	41·0	1,399,185	624·6	61·2	1,885	3·4
Carmarthen	163,772	98,253	60·0	5,348,345	2,387·5	54·4	1,358	0·8
Pembroke	61,556	34,469	56·0	1,976,695	882·5	57·3	2,769	6·1

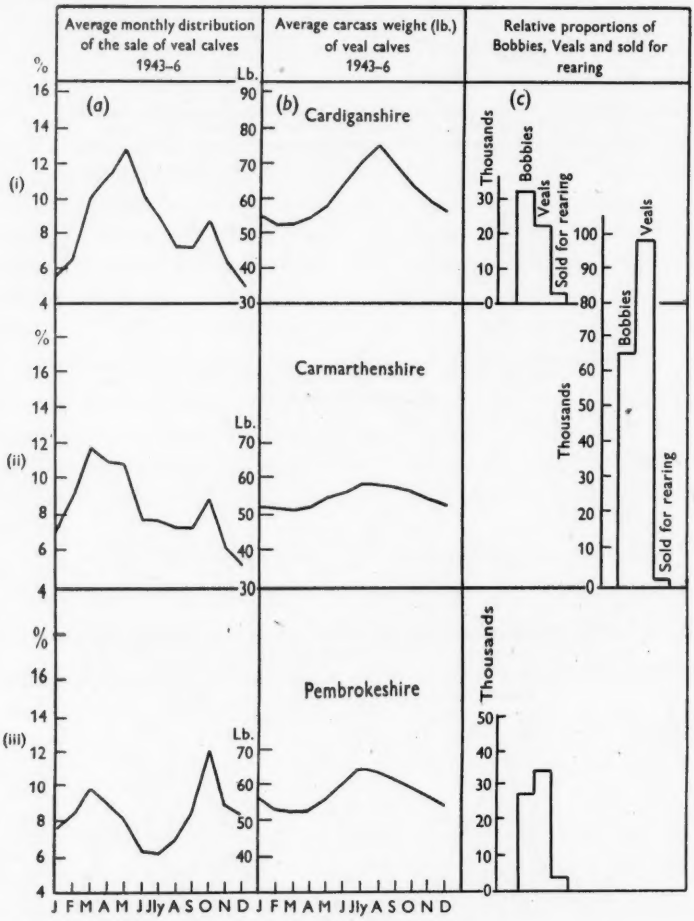


Fig. 4.

2. VEAL PRODUCTION PER COUNTY

(*a*) *General*

A study of Table 3 and Fig. 1*b*, *c* and Fig. 4*c* shows that Carmarthenshire produces 50% more calves and veal calves than the other two counties put together. It has a higher percentage of veal calf sales to total calf sales (60·0%), but shows the least average carcass

weight (54·4 lb.) and also the smallest proportion (0·8%) of calves sold by the Ministry of Food to farmers for further rearing.

Comparing Cardiganshire and Pembrokeshire, the latter county is superior in the volume of its veal trade, in the percentage of veal calves sold and in the proportion sold for rearing; but the former county provides the highest average carcass weight (Fig. 4*b*) and the lowest percentage of vealers. In other words, Table 2 shows that the most intensive milk-producing county of the three (Carmarthenshire), and incidentally the largest county in Wales, supplies the greatest numbers and the highest proportion of veal calves but of the lowest average carcass weight, and it has the lowest number and proportion of calves sold for rearing. Cardiganshire, however, with the smallest number and lowest proportion of veal calves, and of veal, yet provides the highest average carcass weight and Pembrokeshire occupies the intermediate position.

Phillips (5) has shown that Carmarthenshire sells nearly 70% of its annual calf crop whilst the other two had only reached 50% calf crop sales by 1946. Consequently, it appears that Carmarthenshire produces the highest proportion of light-weight veal calves in the greatest numbers, whereas Cardiganshire and Pembrokeshire with more recent tradition in good store-cattle rearing, keep a smaller proportion of their surplus calves so as to attain heavier weight veal calves. This will be brought out later in greater detail when dealing with collecting areas. In all probability, Carmarthenshire produces more Friesian calves, which have been shown to have a heavier birth weight than other calves and consequently can readily be made into vealers at a younger age and at a smaller expense to the farmer.

(*b*) *Annual production of veal calves per county*

Fig. 1*b*, *c* illustrates the extent of the annual increase in the sale of total calves, of veal calves and of tons of veal in each of the three counties. The Carmarthenshire production dominates the picture, with Pembrokeshire slightly ahead of Cardiganshire.

The annual differences in the nature of the production are shown in the following Table 4.

Table 4. *Annual changes in the average carcass weight and in the percentage of veal calves in the three counties*, 1943–6, *together with corresponding average wholesale producers' price per gallon of milk* (*M.M.B.*)

	Cardiganshire		Carmarthenshire		Pembrokeshire		Av. pool-price of milk per gal.
	Percentage veal calves	Av. carcass wt. (lb.)	Percentage veal calves	Av. carcass wt. (lb.)	Percentage veal calves	Av. carcass wt. (lb.)	
1943*	55·5	62·8	59·5	55·9	60·7	59·1	23·5
1944	40·5	61·8	60·2	54·7	57·7	57·4	23·83
1945	36·9	61·6	60·3	54·2	53·0	57·1	24·35
1946	36·6	59·0	59·6	53·4	54·1	54·1	25·73

* The 1943 data for Carmarthenshire do not include five of the nine centres.

Table 3 and Fig. 5*a* show that there has been a gradual lowering of both the percentage of veal calves and the average carcass weight during the 4 years. The Cardiganshire figures show a drop of nearly 20% in veals from 1943 to 1946, whilst the drop in average carcass weight is over 3 lb. in Cardiganshire and Pembrokeshire, but only 2½ lb. in Carmarthenshire in the same period. The drop in average carcass weight corresponds to the increasing pool-price of milk as shown in the above table and illustrates the effect of the varying

prices for milk and the constant price of veal on the average carcass weight. (The Carmarthenshire figures show the lowest average carcass weight and the highest percentage veals.)

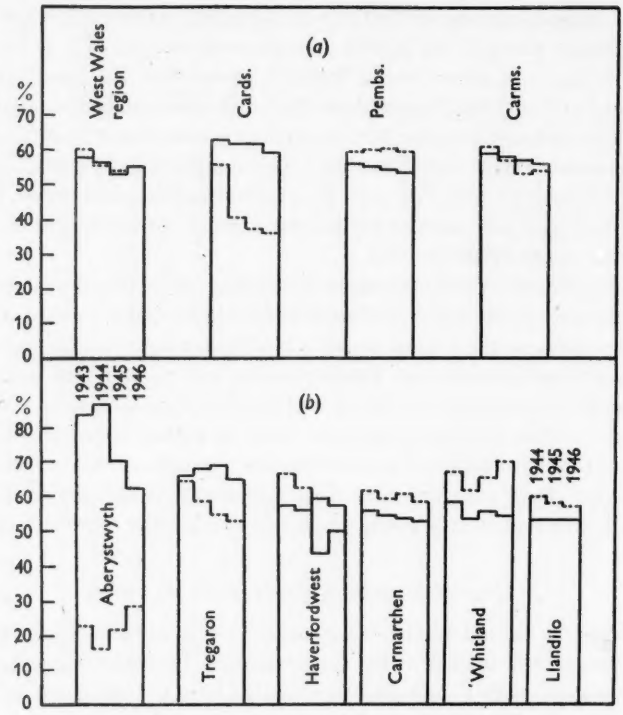


Fig. 5. Annual changes in (*a*) average carcass weights of veal calves (——); (*b*) percentage veals in total calves (- - -).

(*c*) *Seasonal production of veal in the counties*

Average figures of monthly production per county over the 4 years are illustrated in Fig. 6. This brings out county differences—thus Cardiganshire and Carmarthenshire show high spring deliveries and only a minor rise in October, whereas Pembrokeshire shows only a moderate spring peak but a higher October peak. This is in accordance with county differences in total calf sales shown by Phillips & Davies (2) and indicates that the variation in the sale of veal calves from season to season follows the same trend as that of the sale of all calves. The curves for the average carcass weights in the three counties (Fig. 6) show high July/August figures and low spring weights, but whereas there is a difference of 22 lb. between the maximum and minimum in Cardiganshire, it is 12 lb. in Pembrokeshire and only 7–8 lb. in Carmarthenshire, but the latter county is at least remarkable for its level average monthly carcass weight. In the relative proportions of vealers to bobbies (Fig. 4*c*) it is seen that in Cardiganshire there is a higher proportion of bobbies, whilst in the other two counties the order is reversed. In all three counties the proportion sold for rearing is very low; it is, however, highest in Pembrokeshire and lowest in Carmarthenshire.

The relationship between the average monthly sale of veal calves and of veal is shown in

Fig. 7. The monthly percentage of veal calves is higher in the early part of the year; in May/June there is a crossing over, and in the latter half of the year the order of the two percentages is reversed. This means fewer calves of heavier weights in the latter half and the reverse in the early part of the year. It is seen that the differences are greatest in Cardiganshire.

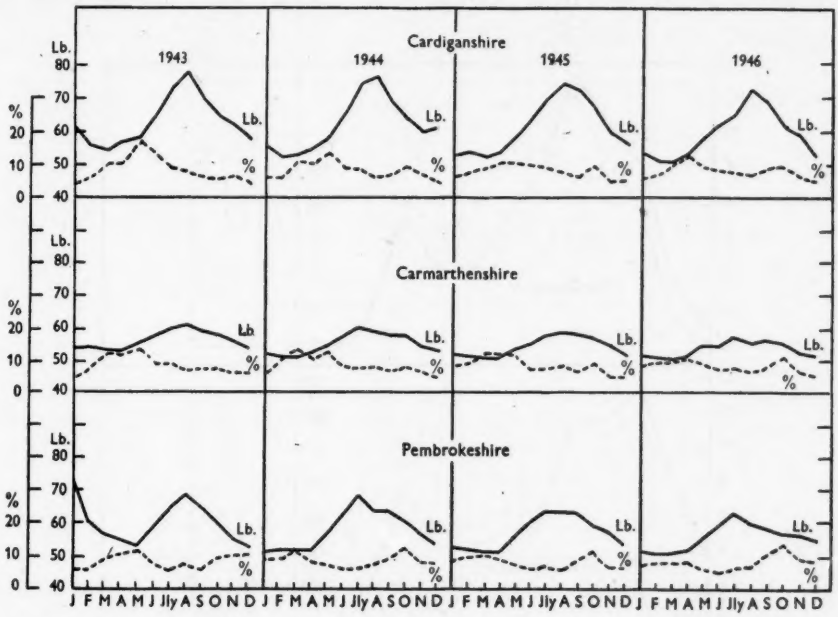


Fig. 6. Average monthly carcass weights and distribution of veal calves each year, 1943–6.

(*d*) *Monthly ratios of the numbers of veal calf sales to sales of all calves* (1943–6)

Fig. 8*d* shows these proportions as 4-year averages. It is seen that the three counties have exactly the same pattern in the graphs, although at different levels; Table 3 shows that Cardiganshire has the lowest annual average of 41·0%, next follows Pembrokeshire with 56% and then Carmarthenshire with the highest at 60%, and at present there is no satisfactory explanation for the repetition of the zig-zag pattern on these three graphs except the one already given for the regional graph (Fig. 3*e*).

(*e*) *Calves sold for rearing* (*county*) 1943–6

As previously shown, relatively small numbers of calves were bought for further rearing by farmers after grading, but Fig. 8*a–c* throws light on certain trends in this connexion:

(1) The monthly distribution of the calves sold for rearing shows very decided peaks between May and June when milk is plentiful, and relatively cheap. The pattern of high summer and low winter purchases for rearing is the same in the three counties, although Carmarthenshire has a slightly higher winter level than the others.

(2) The total annual purchases per county are always regular. Carmarthenshire purchase is always very low, Pembrokeshire high and Cardiganshire intermediate, but on the

whole there is a tendency towards lower percentage purchases during the sequence of the years.

(3) The distribution as far as colour is concerned (Fig. 8*b*) shows that Cardiganshire has definite preference for roans, Carmarthenshire only a slight preference for reds over roans, whilst Pembrokeshire prefers the red and white. These R/W (= Red and White) calves consist very largely of Herefords and Hereford crosses, rather than of red and white Shorthorn. The

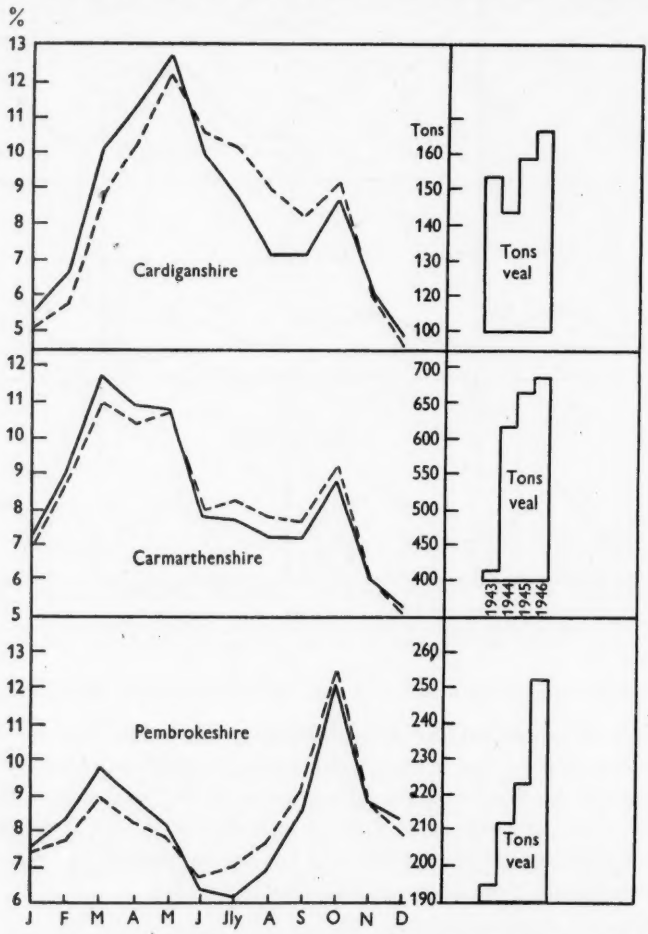


Fig. 7. Seasonality of production of veal and veal calves per county, and veal production in tons. ——, percentage veal calves; - - - -, percentage veal.

first three colours, red, roan and red and white account for nearly 75% of all the purchases in each of the three counties. One general conclusion is, however, that the region is dominated by the Shorthorn breed, although the Hereford and its crosses find favour in Pembrokeshire and the Blacks in Cardiganshire. Very few Friesian coloured calves are purchased. It is unfortunate that the colours of all the calves are not clearly given in the mart returns. It was found impossible to make a satisfactory analysis of them in order to determine the predominating breeds in each collecting centre.

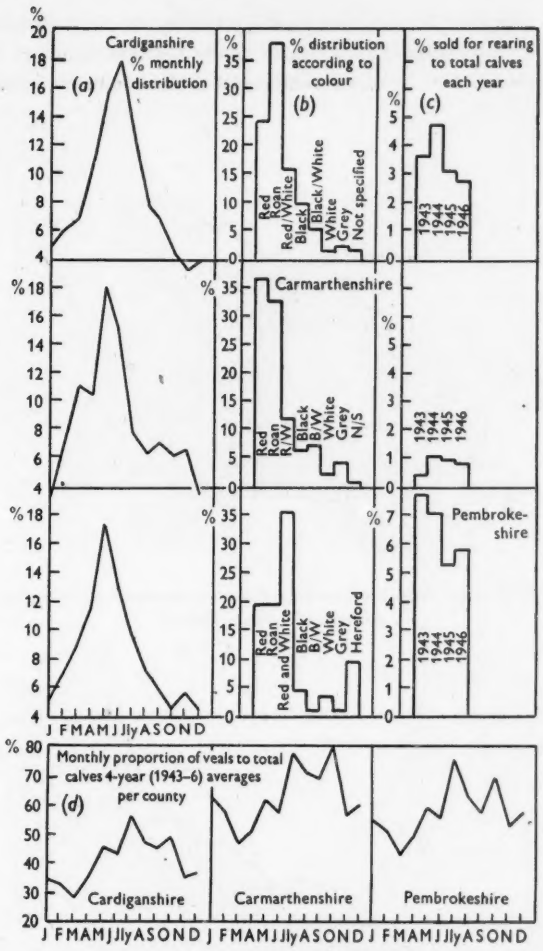


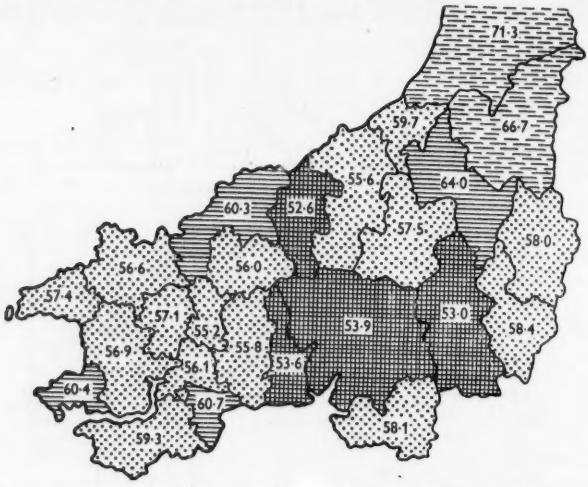
Fig. 8. Calves sold for rearing, 1943–6.

3. VEAL PRODUCTION PER CENTRE

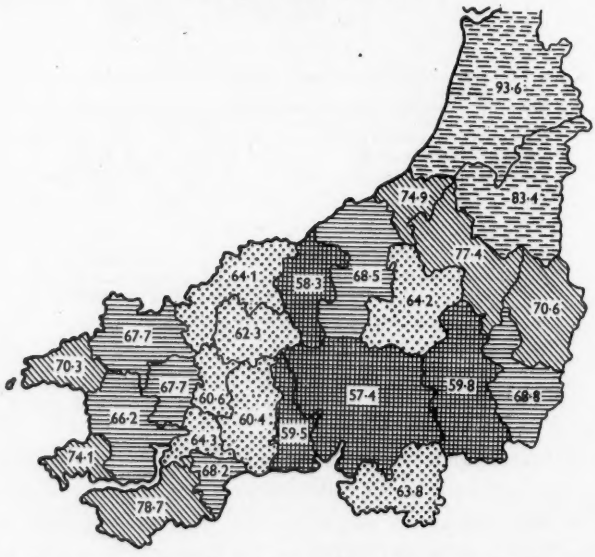
(a) *General*

The volume of veal production varied enormously in the twenty-five collecting centres (Maps 1 and 1A), due to the size of the collecting area, the intensity of dairy farming, and to some extent to the transference of calves from the region of birth to a convenient marketing centre. This is particularly true for the Carmarthen centre, where there is a weekly cow and calf mart which draws its supplies from a wider area than the fat-stock collecting area. Consequently, calves find their way to the Carmarthen centre and, to a lesser extent, to the Lampeter centre from areas outside the particular collecting area in the allocation of parishes. Indeed, during the 4 years under consideration, Carmarthen supplied over 55,000 veal calves (1331·5 tons) at an average carcass weight of 53·9 lb., while, on the other hand, Clarbeston Road supplied only 1907 veal calves during the same period (48·6 tons) at an average carcass weight of 57·1 lb.

Because of the variation in size and in the intensity of dairy farming in the various collecting areas, no valid comparisons can be made between them as far as quantity of production is concerned. On the other hand, very useful conclusions can be derived from



Map 2. Average annual carcass weight of veal calves per collecting centre.
Average of 4 years, 1943–6.



Map 3. Average maximum monthly carcass weight of veal calves per collecting centre.
Average of 4 years, 1943–6.

a study of the nature of production as illustrated by the varying average carcass weights, by the seasonality of those carcass weights and by the seasonality of production.

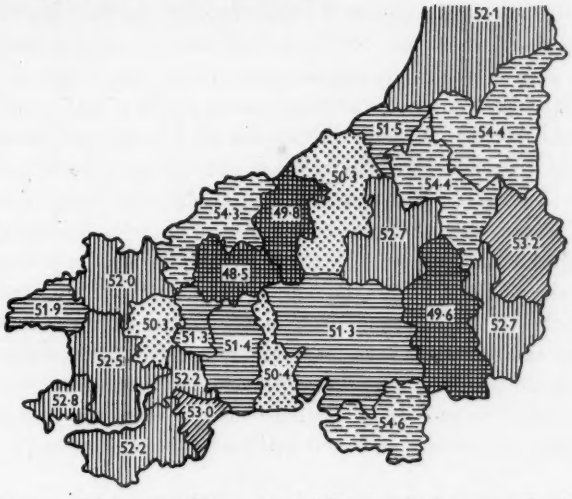
These are illustrated in the maps and figures.

Map 2 gives the average carcass weight per collecting centre during the 4 years.

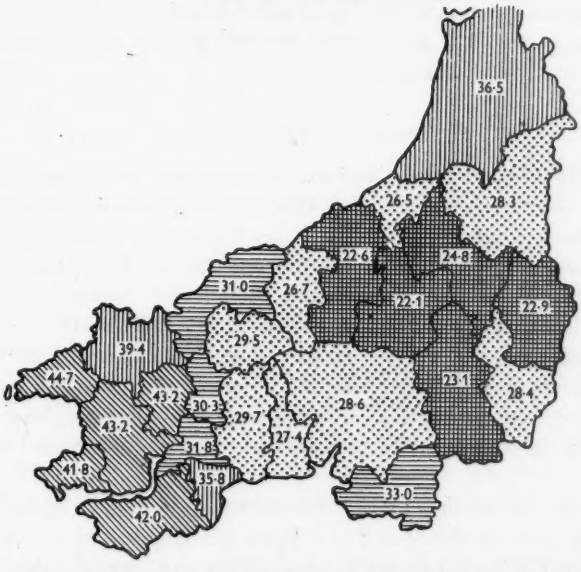
Map 3 gives the maximum monthly average carcass weight (4-year average).

Map 4 gives the minimum monthly carcass weight (4-year average).

Map 5 gives the average autumn (September to December) sales of veal calves per centre as a percentage of total annual calf sales.



Map 4. Average minimum monthly carcass weight of veal calves per collecting centre. Average of 4 years, 1943–6.



Map 5. Average percentage of sales of veal calves during the autumn months (September to December) per collecting centre. Average of 4 years, 1943–6.

The maps, in general, show considerable variation within the region of the three counties between the average figures per centre (Map 2) and maximum monthly carcass weights (Map 3), but with a smaller difference between the minimum monthly carcass weights (Map 4). In all three maps, however, there is the same trend of having the lowest figures

in each case in the main central core of the region, and the higher figures at the extremities in the north, east and west. For example, the Aberystwyth centre in the north-east shows the highest figures in the three maps with Tregaron and Lampeter next in order of magnitude. The western coastal centres of Pembrokeshire also show higher figures than the inland centres, whilst Carmarthen, St Clears, Whitland, Newcastle Emlyn and Llandilo show the low weights of veal calves characteristic of the central part of the region.

In all probability, variation in the average carcass weight of veal calves in the different centres is due to the fact that the central region has the longest tradition in dairying due to the comparatively early advent of the railways and the early development of milk and butter factories (Davies (1)). Consequently, the tradition of good rearing has largely disappeared and surplus calves have to be disposed of either as bobbies or at the value of the most easily attainable veal weight, bearing in mind the supplies of colostrum and post-colostrum milk, size of the calf and the date of the next mart. In contrast, the remote areas were unaffected by early developments in dairying. Dairying in these areas is largely the result of rapid war-time encouragement; consequently a higher proportion of producers have remained cattle rearers, and even those that have gone over to milk-selling still retain some traditional pride in good rearing. Producers in these remote areas are less conscious of the relative prices of milk and veal than those of the central area of intensive dairying.

This provides an example of the time required to effect a change in the system of husbandry in a given area. The distance of a region from railways and depots, together with the inaccessibility of farms in the dales and foothills, make for a slow rate of change from one system to another, and in many respects the old and traditional methods influence farming for a very long time.

(*b*) *The seasonality of production* (*per centre*)

The seasonality of production—both in numbers and in average carcass weights is shown for each collecting centre in Fig. 9. Here it is seen that contrast between the centres is not in the pattern of the graphs, as in all cases the summer figures are higher than those of winter, but rather in the size of the difference between the maximum (July) and minimum (March) figures. In the extremities of the west Wales region in Aberystwyth, Tregaron and Llandovery in the east, in Croesgoch, Hasguard and Pembroke in the west, there is a big difference between the maximum and minimum average monthly carcass weights (also shown in Maps 1–3). This gap is narrowed gradually towards the central part of the region and the centres of Carmarthen, Whitland, St Clears and Newcastle Emlyn show less than 10 lb. difference between highest and lowest average monthly carcass weights.

It is remarkable how adjoining territorial districts show such a constant, albeit gradual, change. This is further illustrated in Map 5 which is reminiscent of the cline in calf sales of the east and west (Phillips & Davies (2)). One possible explanation is that there are factors in farming, other than controlled and systematic husbandry, which influence animal production. The height of the autumn peak in veal calf production in Pembrokeshire (e.g. Haverfordwest) and its absence in the easterly centres (e.g. Llandovery) is reminiscent of the curves for the sales of all calves (Phillips & Davies (2)), where it was suggested that climatic conditions at the time of conception (in winter) have a profound effect on the extent of autumn calving. Robinson (6) has put forward the suggestion that the length

of the breeding season, controlled as it is by pituitary activity, is influenced by environmental conditions of feed and climate.

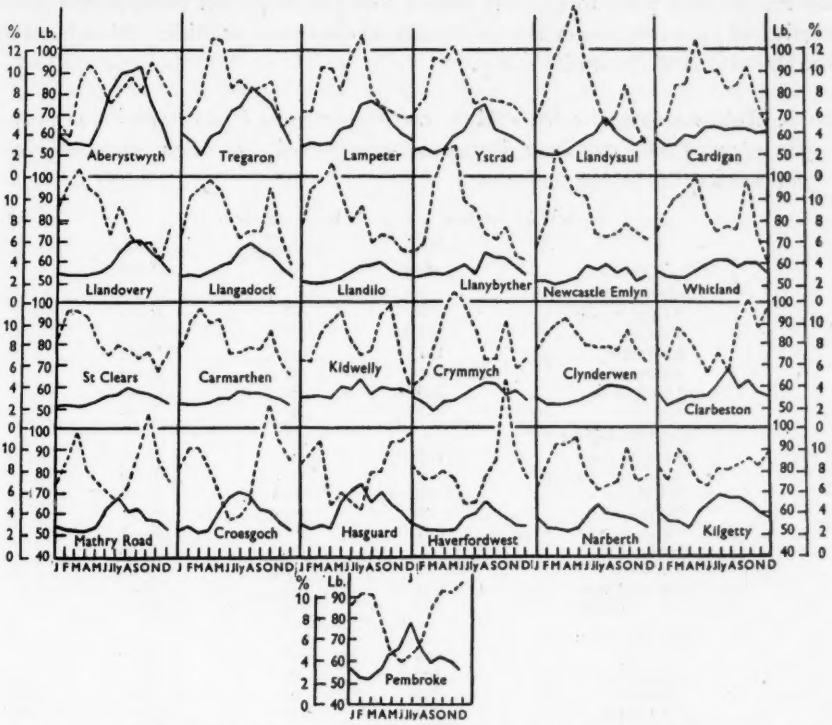


Fig. 9. Average monthly weights of veal calves in pounds and average percentage monthly distribution of veal calves per collecting centre, 1943–6.

(c) *Sales of calves for rearing (per centre)*

Table 5 gives particulars for the four years (1943–6) of the sales of calves for rearing at each centre (except in the case of five Carmarthenshire centres where the 1943 records are missing).

It is seen that the centres, Aberystwyth, Tregaron, Llangadock and Llandovery situated at the eastern end of the region, sell a higher percentage of calves for rearing than the more westerly areas of Cardiganshire and Carmarthenshire, and that the more intensive dairying areas of Pembrokeshire, viz. Narberth and Clynderwen, sell a lower percentage than the more remote areas of the west. This is in general agreement with the picture brought out by Maps 2–4.

The entries under 'Colour' are merely an indication of the preferred colours of calves chosen for rearing. In some cases the choice of particular colours is very definite, but in others the advantage of the red over the roans or vice versa is slight. It is seen that all the Cardiganshire centres prefer roans whereas in Carmarthenshire the proportion is five roans to four reds. In Pembrokeshire the dairying centres of Narberth and Clynderwen prefer roans, as does Crymmych, whilst Pembroke and Kilgetty prefer reds, but the remaining centres, all concerned with rearing, prefer R/W which in all probability are Hereford

crosses. By including the Herefords (entered only in the Pembroke centre) with the R/W this centre also shows a very high preference for this class of beef type calf. The years under review were prior to the calf subsidy and free Beef A.I. Scheme, but it is an indication of the soundness of the policy that farmers were practising this scheme even before the advent of the official policy.

Table 5. *Total numbers of calves sold for rearing per centre* (1943–6) *and expressed as a percentage of total calf sales during the same period. The colour preference for the purchased calves is also indicated*

R = sold for rearing. T = total calf sales.

Centres	Sold for rearing	Percentage R/T	Colour preference
Cardiganshire:			
Aberystwyth	650	6·4	Roan
Tregaron	442	7·7	Roan
Lampeter	187	3·1	Roan
Ystrad	261	3·5	Roan
Llandyssul	106	1·0	Roan
Cardigan	239	1·7	Roan
Carmarthenshire:			
Llanybyther	157	1·9	Roan
Newcastle Emlyn	69	0·37	Roan
Whitland	84	0·84	Roan
St Clears*	30	0·44	Red
Carmarthen	357	0·38	Roan
Kidwelly*	75	2·0	Roan
Llandilo*	87	0·64	Red
Llangadock*	220	5·4	Red
Llandovery*	279	5·2	Red
Pembrokeshire:			
Crymmych	319	5·6	Roan
Clynderwen	140	1·7	Roan
Mathry	747	7·4	R/W
Croesgoch	431	10·9	R/W
Clarbeston	229	7·4	R/W
Hasguard	119	4·1	R/W
Haverfordwest	508	4·5	R/W
Narberth	159	2·5	Roan
Pembroke	659	18·3	Red
Kilgetty	458	8·3	Red

* 1943 records missing. R/W, red and white.

(*d*) *Annual production of veal* (*six centres*)

Six centres, viz. Aberystwyth, Tregaron, Carmarthen, Llandilo, Whitland and Haverfordwest, have been selected for the purpose of examining the annual production of veal.

Fig. 5*b* (page 10) gives the average annual carcass weight of veal calves and the annual percentage veal calves and illustrates the differences between the recent rearing areas and the old dairying areas: e.g. Aberystwyth shows a high average carcass weight but a low percentage of veals.

(*e*) *Seasonality of production of veal* (*six centres*) *each year*

Average monthly carcass weights are shown in Fig. 10 and illustrate the similarity between the annual trends of total veal production and the dissimilarity between productions and individual centres. Three centres, Aberystwyth, Tregaron and Haverfordwest, show large monthly differences each year, whilst at the other centres carcass weights vary within much narrower limits.

The extent of seasonal variation in the number of veal calves sold, particularly in the sales during the autumn months, confirm the conclusion drawn from the previous study of the sale of all calves, namely that there is also an east to west cline in the proportion of autumn veal calves. It has been postulated that this cline is the result of climatic conditions during the months of conception of autumn-born calves, viz. December to March.

On the other hand, the variation in average carcass weight from centre to centre is in all probability a reflexion of husbandry systems. The heavier veal calves are found in the more traditional store-rearing parts and the lighter calves in the more intensive dairying parts of the region.

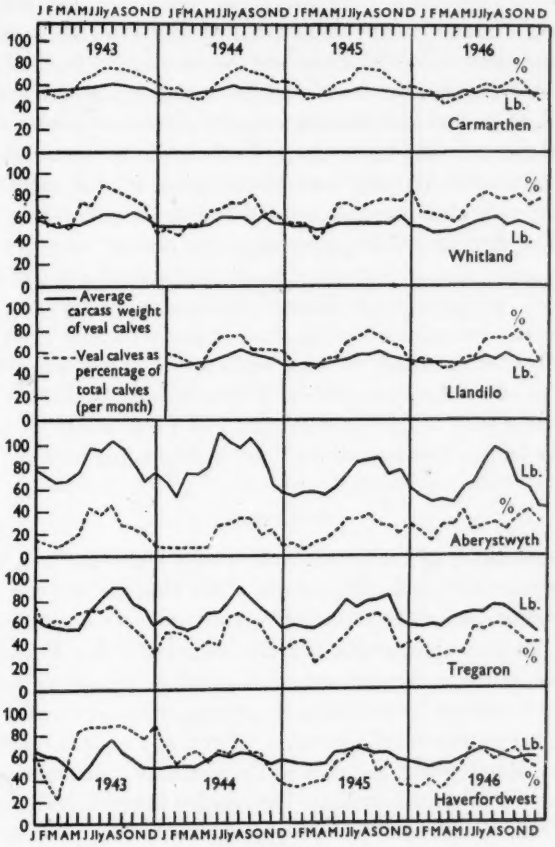


Fig. 10.

CASUALTY CALVES

Included in the records of the mart returns from the collecting centres are the casualty calves which are accepted for slaughter at any time.

These consist of ailing calves as well as those that have suffered damage of some sort or another. Their acceptance for slaughter at a reduced price is the attempt made to salvage these unfortunate animals and to put them out of their misery in the shortest possible time.

The total returns for the casualty calves are given in Table 6.

Table 6. *Total annual number of calves and of casualty calves sold per county, together with the casualty calves as a percentage of total sales* 1943–5

	Cardiganshire			Carmarthenshire			Pembrokeshire		
	Total calf sales	Total casualty calves	Percentage casualty calves	Total calf sales	Total casualty calves	Percentage casualty calves	Total calf sales	Total casualty calves	Percentage casualty calves
1943	11,821	93	0·79	39,793	152	0·38	12,595	69	0·55
1944	12,953	72	0·56	42,110	225	0·53	14,415	80	0·55
1945	15,572	83	0·53	45,587	138	0·30	16,432	70	0·42

The percentage of incidence is fortunately very low. The probability is that many casualty calves would in any case be slaughtered as bobbies or veals, so that the figures cannot be used to measure the extent of losses of calves in early life, although they indicate that within the west Wales region there is very little variation in the percentage of casualties. On the other hand, December and January casualty calves are usually the highest.

It is true that higher casualty figures are to be found in the central parts of the region devoted to more intensive dairying, and lower figures in the more remote districts which have only recently abandoned store-cattle rearing for milk selling. In these typical dairying, milk-selling districts calf life, especially in the case of males, is of very little consequence. There is grave danger that owing to the commercialization of the industry, the care of farm animals, so important in livestock husbandry, particularly during the early stages of life, is rapidly becoming a lost art. Consequently the rearing of calves, including of course heifer-calves, so necessary for herd replacement, is less well attended to than it was in the past. The effect of stingy upbringing is to be found in stunted young cattle and later on in life in lower body weights of those that go for beef and in lowered milk yields of the majority of the heifers that go into the dairy and breeding herds.

SUMMARY

The volume and seasonality of veal production in west Wales has been obtained from an analysis of the records of the collecting centres of the Ministry of Food.

It has been found that about 1000 tons of veal are produced annually from 55% of the calves sold to the Ministry at an average carcass weight of 56 lb. Most of the veal is produced in the season of the maximum number of calvings, i.e. spring in the eastern areas and autumn in the western areas. Higher average carcass weights are produced in summer and a higher proportion of the calves offered, are veals during the summer than during the winter. March might be taken as the month of maximum veal output but of lowest average individual carcass weights. The relation between veal weights, production and the average price of milk has been illustrated, and the conclusion drawn is that when milk is cheap (summer) veal weights are high. The more intensive the dairying (in the central part of the region) the lower the average carcass weight and vice versa for the less intensively dairying areas. Calves sold for rearing are only of minor significance in the calf trade in west Wales, but one consolation is that the incidence of the casualty calf is also slight. The choice of colour in calves purchased for further rearing supports the general opinion that during the period under review (1943–6) the Shorthorn breed was the most important, and its Hereford crosses the most popular choice in the more remote beef-producing areas of Pembrokeshire.

In the centres situated in the two main river valleys, viz. the Teify and the Towy, the

average carcass weight of veal calves falls as one proceeds from the foothills to the estuary. This again may be taken as a measure of the degree of intensity of the dairying system and maybe of the consequent loss of care and attention towards young animals. Taking veal weights as an index it would appear that the geographic suitability of an area for dairying spells the doom of good rearing. The same is true of the economic and financial returns of the two systems—dairying and rearing. The need for better returns on poor land leads eventually to milk selling and to poor rearing, but the old tradition of good rearing dies hard and it is to be hoped that the calf subsidy scheme will help to maintain it.

This investigation has formed part of the survey of animal health and production in west Wales, and thanks are due to the scientific branch of Messrs Cooper McDougall and Robertson, Berkhamsted, for financial support. Grateful acknowledgement is also made to the Ministry of Food (Meat and Livestock Branch), to Major John Francis and to the Milk Marketing Board for permission to examine the records; and to Messrs R. Jones and Ll. R. Evans for their work in collating the material; finally our grateful thanks to Miss Joan Addyman for her interest and assistance in helping to prepare this and many other papers.

REFERENCES

(1) DAVIES, A. J. (1939). Unpublished Thesis. University of Wales.
(2) PHILLIPS, R. & DAVIES, J. L. (1949). *J. Dairy Res.* **16**, 1.
(3) PHILLIPS, R. (1946). *Nature, Lond.*, **158**, 202.
(4) WALLACE, L. R. (1948). *J. agric. Sci.* **38**, 93, 243 and 367.
(5) PHILLIPS, R. (1947). Unpublished data.
(6) ROBINSON, T. J. (1949). Unpublished Thesis. University of Cambridge.

(*MS. received for publication* 8 *December* 1949)

636.2:612.664.31

407. THE NUMBER OF SWEAT GLANDS IN THE HELIX OF THE COW'S EAR AND THE MILK YIELD

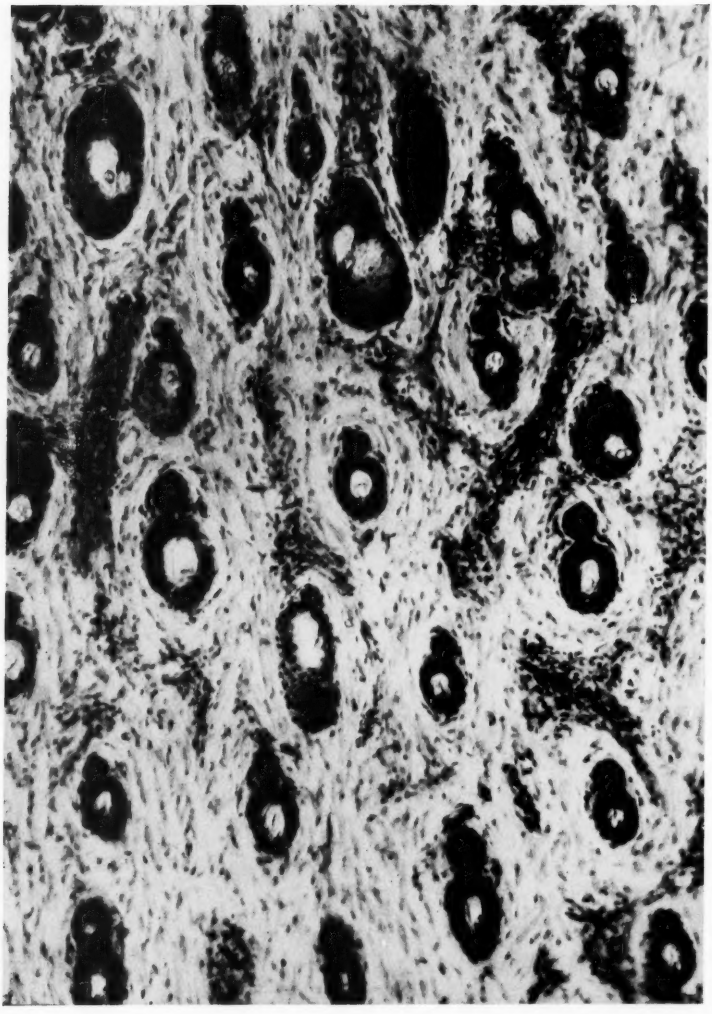
By J. D. FINDLAY, A. MYFANWY GOODALL and S. H. YANG
The Hannah Dairy Research Institute, Kirkhill, Ayr

(With 1 Plate and 2 Text-figures)

The possibility of a relationship between the histological structure of the mammary glands and the milk production of dairy cattle has been investigated by various workers (Liskin (1), Nemilov (2), Pesciscev (3), Cingovatov (4), Lisick (5), Simic (6), Okaladnov (7), Podoba (8)). It is obviously impracticable to make a histological study of the mammary tissue of living cows in order to assess their milk-producing capacity, but Nemilov (9) claimed that there was a correlation between the development of the 'sweat glands' in the skin of the helix of the ear and the amount of glandular tissue in the udder. This correlation was explained by him on the basis of the close phylogenetical relationship between the mammary glands and the 'sweat glands' of the cow. Since then, other workers have endeavoured to find what correlation there is in various breeds of cattle between the milk yield and the number of sweat glands per unit area of skin in the ear (Zamjatin (10), Konjkov (11), Podoba (8), (12), Krasnokutsk (13), Burcev (14)). With the exception of Krasnokutsk (13), they all found a significant positive correlation.

The distribution of 'sweat glands' in the bovine ear might well be expected to vary from one area to another, but from a study of ten right and ten left ears of cows of Red German breed, Burcev (14) claimed that there was no difference in the number of sweat glands per unit area of skin between the two ears or between different regions of the same ear. He concluded that samples of skin from any region of the ear could be used for obtaining the sweat-gland count, and that the number so obtained would be representative of any other region of either the left or right ear. Furthermore, as a result of an investigation involving 1336 Red German cattle of both sexes and various ages, he claimed that the number of sweat glands per unit area of skin in the ear did not depend on either age or sex but was characteristic of the particular animal being studied.

If the claims made by these various workers, and particularly by Burcev, were substantiated for any of the principal breeds of dairy cattle, a relatively simple method of great value in selecting cattle for high milk production would immediately become available. The experiments described in the present paper were therefore made to determine whether Burcev's findings for Red German cattle applied also to Ayrshire cattle. Three aspects of the problem received particular attention. First, the distribution of the sweat glands in the ear was studied in detail to determine whether Burcev's conclusion was valid that any portion of either ear could be used to obtain the sweat-gland count, and that the count so obtained would be representative for any other region of either ear. Secondly, an experiment was made to determine whether Burcev's claim could be confirmed that the sweat-gland count is independent of the age of the animal, and is the same for the very young calf as it is for the adult cow. Thirdly, attempts were made to find what correlation there is between the sweat-gland count and the milk yield.



Horizontal section of skin from the pinna of the ear of an Ayrshire cow, showing the hair follicles and accompanying sweat gland ducts. (Stained Hx-Eosin).

Throughout the present paper the term 'sweat gland' has been adopted, since the gland under discussion has been referred to in this way by the various workers cited. It should be noted, however, that its adoption by the present authors does not necessarily imply that they regard these glands as true sweat glands. Their nature is discussed elsewhere (Findlay & Yang (15)).

EXPERIMENTAL

(1) *The distribution of the sweat glands in the helix of the ear*

Previous studies on the distribution of sweat glands in cattle skin have shown that each sweat gland and its duct are accompanied by one hair, so that to count the sweat glands in a given area of skin, it is necessary only to count the hair follicles (Findlay & Yang (15), Yamane & Ono (16), Yang (17)), and it can be seen from Pl. 1 that this also holds for the skin of the ear. In fact, if attempts are made to count the sweat glands themselves, the procedure which has been adopted by Burcev (14), large errors may arise owing to the presence of convolutions which may cause one gland to appear several times in one section. Counting the hair follicles avoids this difficulty and introduces no additional errors.

Two methods were adopted in the present work for studying the distribution of the sweat glands. In the first method the hair follicles were counted in carefully prepared sections of the skin taken from different regions of the ear, but since it was realized that the preparation of the sections would lead to some contraction and possibly to distortion of the skin, the distribution was investigated also by counting the hairs themselves on the shaved skin of an ear which had just been amputated from an animal immediately after slaughter. The results obtained by each of these methods are recorded briefly below.

The distribution obtained by counting the hair follicles in sections of the skin. Six different regions of both the right and left ears of a cow were chosen, *A*, *B*, *C*, *D*, *E* and *F* as shown in Text-fig. 1. Two adjacent pieces of skin were cut from each region a short distance from the edge of the ear and fixed in 10% formalin. Sections parallel to the skin surface, and 50 μ thick, were cut by means of a freezing microtome, and were stained with toluidine blue, mounted, dehydrated and cleared in the usual way. The images of the sections were projected on white paper using a microscope whose field covered an area on the section of 20 sq.mm. Only those sections of skin near the surface level were used for counting, and ten counts in all were made from the sections from any one piece of skin. The results were averaged and expressed as the number of sweat glands per sq.cm. of skin surface area. The data thus obtained, with the analysis of variance, are given in Table 1. It can be seen from the table that the variance between the right and left ears was significant while the variances due to regions, and to the interactions of region and surface and ear and region, were highly significant. The variance between the outer and inner surfaces, however, was not significant. It follows therefore that in attempting to determine the sweat-gland count very different counts will be obtained in the Ayrshire cow depending on the particular ear and region of the ear which is chosen. This is contrary to the findings of Burcev (14) for Red German cattle.

The distribution by counting the hairs on an amputated ear. The hairs on a freshly cut left ear taken from an adult Ayrshire cow were clipped and the ear divided into three portions, as shown in Text-fig. 1; an upper margin *ABD*, a lower margin *ACE* and a central region. The hairs of the ear were shaved sufficiently to facilitate the counting of the individual hairs. The counts were made with a magnification such that the square field covered by the

Table 1. *The number of hair follicles per sq.cm. of skin in different regions of the right and left ears of an Ayrshire cow, with an analysis of variance*

The counts were obtained from sections prepared as described on pp. 23 and 24. The positions of the various regions *A* to *F* are shown in Text-fig. 1. Two adjacent samples were taken from each region.

		Right ear			Left ear		
		Surface (average of 10 counts)			Surface (average of 10 counts)		
Region of the ear	Sample	Inner	Outer	Total of the four averages	Inner	Outer	Total of the four averages
A	1	990	240	2,392	1,499	204	3,087
	2	958	204		1,098	286	
B	1	411	771	2,363	668	948	2,927
	2	287	894		458	853	
C	1	787	209	1,924	661	185	1,729
	2	684	244		638	245	
D	1	1,188	1,767	5,406	451	1,380	3,337
	2	620	1,831		223	1,283	
E	1	205	194	1,093	345	292	1,428
	2	495	199		489	302	
F	1	1,261	968	4,450	706	610	3,040
	2	1,103	1,118		814	910	
Total		8,989	8,639	17,628	8,050	7,498	15,548

Analysis of variance

Source of variation	Degrees of freedom	Sums of squares	Mean squares
Main effects:			
Ear (E)	1	90,134	90,134*
Region (R)	5	3,350,298	670,059**
Surface (S)	1	16,950	16,950
First order interactions:			
ER	5	812,395	162,479**
RS	5	4,227,248	845,449**
ES	1	850	850
Second order interactions:			
ERS	5	73,026	14,639
Error	24	455,820	18,991
Total	47	9,026,901	

		Right ear		Left ear	
Source of variation	Degrees of freedom	Sums of squares	Mean squares	Sums of squares	Mean squares
R	5	3,359,532	671,906**	803,161	160,632**
S	1	5,104	5,104	12,696	12,696
RS	5	1,897,162	379,432**	2,403,293	480,658**
Error	12	253,959	21,163	204,336	17,028
Total	23	5,515,757		3,423,486	

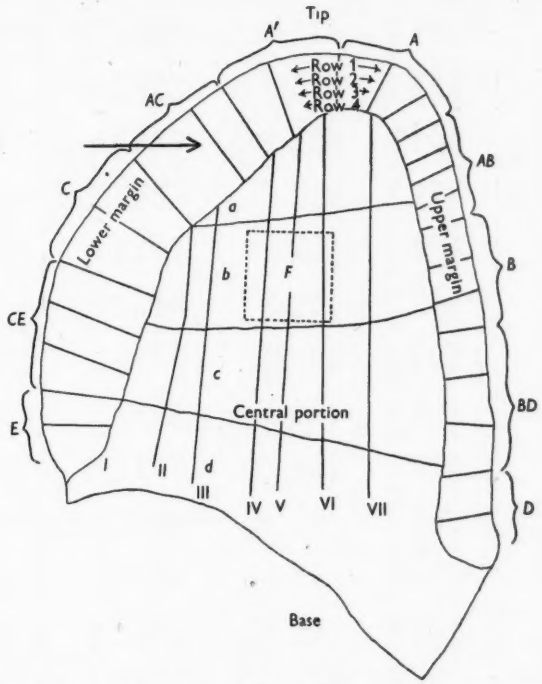
* Significant, $P<0{\cdot}05$. ** Highly significant, $P<0{\cdot}01$.

eyepiece graticule represented 20·25 sq.mm. of the ear surface. A Pointolite lamp was used for illumination. The results quoted in Table 2 will help to illustrate the method adopted and the type of counts obtained. The upper and lower margins of the outer and inner surfaces of the ear were first studied. The figures in any one column of Table 2 show the number of hairs per sq.cm. from the edge of the ear inwards in adjacent fields, i.e. rows 1, 2, 3 and 4 in Text-fig. 1. Along the rows, the counts were also made in adjacent fields, except for short breaks between the main divisions, e.g. in passing from *A* to *AB* or from *AB* to *B* and so on (Table 2 and Text-fig. 1).

It will be seen from Table 2 that in the region of the first three rows there was a marked

tendency for the number of hairs to decrease from the outer edge of the ear inwards, and in rows 2 and 3 of the outer surface of the upper margin the numbers tended in general to increase from the tip of the ear to the base, but this was not so for the inner surface. On the outer surface of the lower margin there was little tendency for the counts to change from tip to base, but they were again higher at the edge (row 1) than farther towards the centre. On the inner surface of the lower margin the counts tended to increase from the tip to region *C* and then to decrease again towards the base.

To obtain counts for the central portion of the ear, the centre was divided into four rows *a*, *b*, *c* and *d*, and seven columns, I–VII, as shown in Text-fig. 1. The inner surface of the



Text-fig. 1. The locations, in the cow's ear, of the columns, rows, regions and margins in which counts were made.

cow's ear has four cartilaginous ridges and these corresponded with columns I, III, V and the borderline dividing the upper margin from the central portion of the ear. Successive fields were counted in columns I–VII with very short breaks occurring between rows *a*, *b*, *c* and *d*. From the results which are shown in Table 3, it is clear that on the outer surface of the central portion there was a general tendency for the counts to decrease in the first few rows near the tip of the ear and then to increase towards the base. In the inner surface the same general tendency existed, but it was much less marked.

It has already been pointed out that according to Burcev (14) any portion of the ear of Red German cattle can be used to obtain a reliable count of the number of sweat glands per unit area in the ear, but the present work shows very clearly that for the ear of an Ayrshire cow the distribution of sweat glands varied greatly in different regions. For the

Table 2. *Hair counts per sq.cm. for the outer and inner surfaces of the upper and lower margins of the left ear of an Ayrshire cow*

The position of the various columns and rows can be seen in Text-fig. 1

Row	Col. *A*		Col. *AB*				Col. *B*						Col. *BD*						Col. *D*				Sum
	Upper margin—outer surface																						
1	924	1,050	1,410	1,570	1,490	1,550	1,420	1,270	1,684	1,674	1,468	1,290	1,042	1,068	1,095	1,354	1,300	1,115	1,057	1,190	1,304	1,330	28,645
2	420	365	405	450	509	523	558	702	835	858	904	938	790	845	903	938	948	948	1,073	938	929	1,214	16,993
3	252	232	385	360	425	480	510	640	627	701	845	691	587	700	745	854	696	839	953	988	860	800	14,170
Sum	1,596	1,647	2,200	2,380	2,424	2,553	2,488	2,612	3,146	3,233	3,217	2,909	2,419	2,613	2,743	3,146	2,944	2,902	3,083	3,116	3,093	3,344	59,808
	Upper margin—inner surface																						
1	1,348	1,269	1,304	1,198	1,334	1,056	820	726	790	864	815	665	627	860	730	746	680	716	982	977	691	558	19,756
2	711	720	593	805	529	489	493	474	420	449	479	504	404	465	499	390	360	331	913	924	259	237	11,446
3	523	504	553	519	459	355	370	430	360	435	470	401	355	395	355	430	376	266	899	933	247	197	9,832
Sum	2,582	2,493	2,450	2,522	2,322	1,900	1,683	1,630	1,570	1,748	1,764	1,570	1,386	1,720	1,584	1,566	1,416	1,313	2,794	2,834	1,195	992	41,034

	Col. *A*			Col. *AC*						Col. *C*			Col. *CE*						Col. *E*				Sum
	Lower margin—outer surface																						
1	859	602	592	592	663	573	624	573	701	513	612	790	735	943	914	1,017	737	913	864	682	637	523	15,659
2	351	316	286	296	247	227	183	306	291	177	874	301	330	350	276	380	356	281	291	326	276	321	7,042
3	261	301	281	227	192	291	222	326	227	143	143	177	266	202	207	227	227	192	168	187	192	222	4,881
4	237	247	242	207	207	192	197	261	247	187	163	212	177	163	202	212	271	187	163	222	276	276	4,748
Sum	1,708	1,466	1,401	1,322	1,309	1,283	1,226	1,466	1,466	1,020	1,792	1,480	1,508	1,658	1,599	1,836	1,591	1,573	1,486	1,417	1,381	1,342	32,330

	Col. *A*		Col. *AC*				Col. *C*						Col. *CE*						Col. *E*				Sum
	Lower margin—inner surface																						
1	987	864	1,095	1,052	1,023	1,097	1,280	1,252	1,034	978	1,166	1,170	953	987	888	948	840	825	652	558	593	286	20,528
2	677	667	770	824	854	884	1,120	933	760	593	673	667	662	518	385	316	281	311	326	257	212	158	12,848
3	597	642	677	736	750	850	1,028	849	652	523	597	587	513	523	247	267	168	207	163	163	118	123	10,980
4	563	622	597	563	690	637	1,102	809	636	568	513	533	583	437	449	380	331	351	252	261	172	192	11,241
Sum	2,824	2,995	3,139	3,175	3,317	3,468	4,530	3,843	3,082	2,662	2,949	2,957	2,711	2,465	1,969	1,911	1,620	1,694	1,393	1,239	1,095	759	55,597

Table 3. *Hair counts per sq.cm. for the outer and inner surfaces of the central portion of the ear*

The position of the various columns and rows can be seen in Text-fig. 1

Row	Column I	II	III	IV	V	VI	VII	Sum
				Outer surface				
a	—	—	296	306	642	459	390	
	—	—	—	252	385	420	435	5,223
	—	—	—	107	381	385	—	
	—	—	—	—	340	425	—	
b	—	272	320	242	370	415	400	
	—	257	285	350	360	474	479	
	—	—	306	370	439	385	469	11,683
	—	—	266	380	430	375	430	
	—	—	375	434	425	494	504	
	—	—	385	—	—	508	484	
c	395	276	370	533	548	587	587	
	464	266	434	548	583	652	652	
	760	286	568	662	513	716	770	
	390	326	681	691	681	1,140	933	21,532
	—	464	—	—	696	790	844	
	—	—	—	—	850	—	899	
	—	—	—	—	—	—	977	
d	682	667	720	583	790	1,220	919	
	741	617	642	677	878	982	947	17,349
	780	637	741	741	662	1,000	908	
	—	—	—	815	—	—	—	
Sum	4,212	4,068	6,389	7,691	9,973	11,427	12,027	55,787
				Inner surface				
a	—	—	—	805	755	721	415	
	—	—	—	731	666	577	518	6,955
	—	—	—	—	666	617	484	
b	—	741	603	652	677	622	474	
	—	607	568	587	617	671	494	
	—	—	568	617	647	671	568	18,895
	—	—	563	613	617	543	517	
	—	—	568	647	617	578	558	
	—	—	592	691	672	735	—	
c	737	583	617	563	736	775	558	
	597	553	647	582	701	825	642	
	548	597	587	612	721	850	593	
	—	686	642	622	775	1,010	434	26,486
	—	647	587	637	795	1,040	499	
	—	—	—	671	751	—	632	
	—	—	—	—	677	—	737	
	—	—	—	—	—	—	1,020	
d	696	751	667	810	736	849	661	
	681	800	637	755	780	825	726	15,668
	766	785	755	830	751	726	681	
Sum	4,025	6,750	8,601	11,425	13,357	12,635	11,211	68,004

various sets of results obtained in this section of the investigation (Tables 2 and 3) an analysis of variance was made. The results are shown in Table 4. It is clear that, except for one set of columns, those of the outer surface of the lower margin, the differences between counts when analysed in rows and columns were statistically significant.

Table 4. *An analysis of variance of the results recorded in Tables 2 and 3*

	Region of the ear	Source of variation	Degrees of freedom	Sums of squares	Mean squares
1	Upper margin, outer surface	Total	65	8,555,716	—
		Rows	2	5,342,944	2,671,472**
		Columns	21	1,632,256	77,726*
		Error	42	1,580,516	37,631
2	Upper margin, inner surface	Total	65	5,273,609	—
		Rows	2	2,038,957	97,093**
		Columns	21	2,577,983	1,288,991**
		Error	42	656,669	15,635
3	Lower margin, outer surface	Total	87	4,546,441	—
		Rows	3	3,634,197	1,211,399**
		Columns	21	187,517	8,929
		Error	63	724,727	11,504
4	Lower margin, inner surface	Total	87	8,010,293	—
		Rows	3	2,756,080	918,693**
		Columns	21	4,815,891	229,328**
		Error	63	438,322	6,957
5	Central portion, outer surface	Total (for both columns and rows)	100	4,991,829	—
		Columns	6	804,039	134,007*
		Error	94	4,187,790	44,510
		Rows	3	2,600,976	866,992**
		Error	97	2,390,853	24,648
6	Central portion, inner surface	Total (for both columns and rows)	101	1,279,931	—
		Columns	6	275,874	45,979**
		Error	95	1,004,057	10,569
		Rows	3	252,287	84,095**
		Error	98	1,027,644	10,486

* Significant, $P<0{\cdot}05$. ** Highly significant, $P<0{\cdot}01$.

(2) *The number and distribution of sweat glands in the ear of a calf*

It has already been pointed out that, according to Burcev (14), the number of 'sweat glands' per unit area in the ear is unaffected by the age of the animal. Burcev studied a large group of animals consisting of 515 cows, 227 bulls and 594 young cattle, the ages of the animals ranging from 1 day to 16 years. Age certainly did not appear to affect the count. Thus for twenty-five calves 1–10 days old, he obtained an average figure of $4{\cdot}72 \pm 0{\cdot}209$ sweat glands per sq.mm. ear surface, and very similar figures of $4{\cdot}71 \pm 0{\cdot}162$, $4{\cdot}71 \pm 0{\cdot}260$ and $4{\cdot}70 \pm 0{\cdot}196$ for animals of 6–9 months, 3–4 years and 10–11 years respectively. Values for the other age groups varied irregularly with age from $4{\cdot}06 \pm 0{\cdot}210$ to $5{\cdot}08 \pm 0{\cdot}198$.

Theoretically it might be expected that the count per unit area would remain unaffected by age once the animal was fully grown, but it seems difficult to explain how in an ear that is growing and constantly increasing in surface area the number of 'sweat glands' per sq.cm. can remain unchanged. A detailed study was therefore made of the ear of a 7-day-old calf. The ear was clipped and shaved, and the hairs counted by the same technique that was used for the cow. The counts were made in the columns shown in Text-fig. 2 and are recorded in Table 5. The columns were numbered I to IX and, as judged by the position of the cartilaginous ridges in the two ears, columns I–VI in Text-fig. 2 for the calf corresponded to columns I–VI in Text-fig. 1 for the cow, but the region corresponding to column VII in the cow's ear was too narrow in the calf for counts to be made. Column VII, therefore, does not appear in Text-fig. 2 and Table 5. An analysis of variance (Table 6)

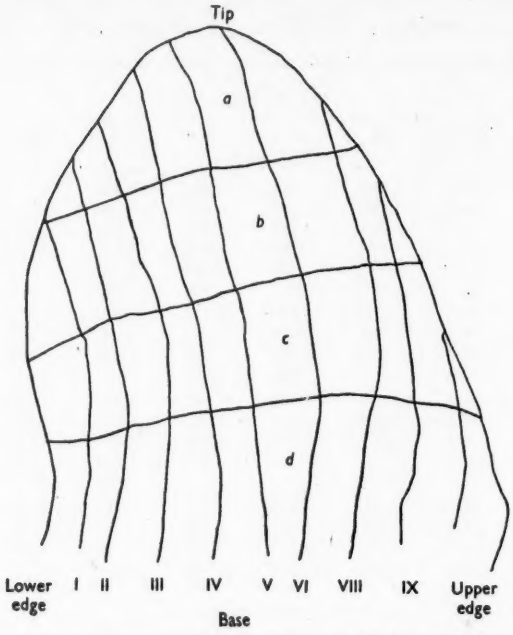
Table 5. *The hair counts per sq.cm. for the outer and inner surfaces of the left ear of an Ayrshire calf*

Text-fig. 2 shows the position of the columns, and the omission of column VII is explained on p. 28.

Row	Column I	II	III	IV	V	VI	VIII	IX	Sum
				Outer surface					
a	—	1,260	1,131	918	1,334	1,398	—	—	
	—	686	736	677	825	859	—	—	
	—	—	741	746	780	958	—	—	20,545
	—	—	—	686	904	1,057	—	—	
	—	—	—	790	943	1,161	—	—	
	—	—	—	—	859	1,096	—	—	
b	904	726	800	933	1,111	1,146	1,300	—	
	760	603	691	904	1,032	1,101	1,328	—	25,567
	746	632	731	909	988	1,096	1,452	—	
	731	820	805	938	1,062	—	1,318	—	
c	558	810	1,027	1,002	899	1,136	1,719	1,472	
	509	672	1,131	988	1,047	1,126	1,694	1,556	
	598	701	1,077	1,017	1,180	1,091	1,141	1,541	35,544
	—	—	—	—	953	1,259	1,343	1,511	
	—	—	—	—	—	1,304	1,704	1,778	
d	1,007	943	1,280	1,146	938	1,067	1,121	1,190	
	1,022	978	1,190	1,057	983	1,363	978	1,254	25,581
	—	1,180	1,210	1,165	1,220	1,244	889	1,156	
Sum	6,835	10,011	12,550	13,876	17,058	19,462	15,987	11,458	107,237
				Inner surface					
a	—	1,773	1,462	2,090	2,341	1,679	—	—	
	—	1,610	1,363	1,590	1,644	1,625	—	—	
	—	—	1,422	1,215	1,323	1,452	—	—	
	—	—	1,190	1,052	1,195	978	—	—	32,494
	—	—	—	988	938	928	—	—	
	—	—	—	—	854	1,022	—	—	
	—	—	—	—	760	—	—	—	
b	1,319	1,002	1,096	1,074	1,240	1,289	884	—	
	1,082	805	1,077	1,081	1,249	1,096	785	—	
	1,017	978	933	1,052	1,328	1,062	657	—	31,558
	894	933	1,086	1,146	1,294	1,215	696	—	
	—	—	—	—	1,215	973	—	—	
c	1,047	1,304	1,126	1,501	1,674	1,121	662	617	
	1,067	1,294	1,072	1,462	1,615	1,254	667	528	
	1,077	978	844	1,348	1,575	1,496	1,007	548	39,339
	923	894	919	1,358	1,531	1,580	1,250	736	
	—	—	—	—	1,783	1,481	—	—	
d	1,091	1,427	1,274	1,536	1,763	1,516	1,294	1,185	
	1,032	1,546	1,175	1,827	1,704	1,788	1,348	993	34,979
	1,200	1,600	1,467	1,901	—	2,040	1,432	1,151	
	—	1,689	—	—	—	—	—	—	
Sum	11,749	17,833	17,506	22,221	27,026	25,595	10,682	5,758	138,370

showed that in the ear of the calf, as in the cow, the number of hairs and therefore of sweat glands per unit area varied significantly from one region to another.

In order to obtain comparable counts for the cow's and calf's ears columns I–VI of Tables 3 and 5 were used, and since the columns in Table 3 represented only the central portion of the cow's ear, additional counts were made so that these particular columns were extended into the marginal areas to make them comparable with the columns used in the calf's ear. From the analysis of variance in Table 6 it is clear that the differences between the counts for the calf and the cow were highly significant. When the various counts for each animal were averaged it was found that the average number of sweat glands



Text-fig. 2. The locations, in the calf's ear, of the columns and rows in which hair counts were made.

Table 6. *Analysis of variance for the results recorded in Table* 5 *for the ear of a calf (analyses nos.* 1 *and* 2), *and a comparison of the counts obtained for the calf with those for the cow (analyses nos.* 3 *and* 4)

Description and number of analysis	Source of variation	Degrees of freedom	Sums of squares	Mean squares
1. The outer surface of the left ear of a calf	Total for both columns and rows	102	7,417,534	—
	Columns	7	3,952,142	564,591**
	Error	95	3,465,392	36,478
	Rows	3	953,685	317,895**
	Error	99	6,463,849	65,291
2. The inner surface of the left ear of a calf	Total for both columns and rows	111	13,231,230	—
	Columns	7	3,638,240	519,748**
	Error	104	9,592,990	92,240
	Rows	3	2,739,173	913,057**
	Error	108	10,492,057	97,147
3. Comparison of the hair counts obtained for the outer surface of the calf's ear with those obtained for the outer surface of the cow's ear	Total	185	17,907,059	—
	Between cow and calf	1	10,329,130	10,329,130**
	Error	184	7,577,929	41,184
4. Comparison of the hair counts obtained for the inner surface of the calf's ear with those obtained for the inner surface of the cow's ear	Total	194	28,669,972	—
	Between cow and calf	1	16,761,887	16,761,887**
	Error	193	11,908,085	61,670

** Highly significant, $P<0{\cdot}01$.

per sq.cm. on the outer surface of the ear of the calf was 973 and for the cow 498, which gives a ratio of 1·98 to 1·0. The corresponding figures for the inner surface were 1297 and 710, giving a ratio of 1·83 to 1·0. The figure of 498 per sq.cm. for an Ayrshire cow is very close to Burcev's average of 482 per sq.cm. for a large number of cattle of the Red German breed, but the fact that the value for the calf was practically twice as great as that for the cow is completely at variance with the claims of Burcev. Nevertheless, the present finding would seem to be the more reasonable, because simple measurement has shown that the surface area of the ear of a very young calf is roughly half that of a cow, and since it is practically impossible to suppose that the total number of the sweat glands in the ear varies during growth, the number per unit area would be expected to decrease during growth.

The results which are recorded above in sections 1 and 2 have been obtained by an extensive study of one cow's ear and one calf's ear, but results obtained by less detailed examinations of the ears of other animals of the Ayrshire breed confirm the general conclusions which have been drawn.

(3) *The relation between the sweat gland count and the milk yield*

Experiment 1. The main object of the present work was to determine whether by adopting the procedure of Burcev (14) the sweat-gland count could be used as an indication of the milk-producing capacity of Ayrshire cows. Burcev claimed that any part of the ear could be used. In a preliminary test, therefore, his method was followed as closely as possible, and although the pieces of ear used to obtain the counts came mainly from the tips of the pinnae of the right ears in the region marked *A* in Text-fig. 1, no attempt was made to take the pieces from precisely the same place for each of the cows involved. Two herds of Ayrshire cattle were used, the Hannah Institute herd which was denoted herd A and that of a nearby farm, herd B. Pieces of skin each 2 cm. in diameter were cut with a branding punch, a method recommended by Burcev (14). The inner surface of each sample was clipped for identification and the pieces fixed in 10% formol saline for 72 hr. Sections 50μ thick, parallel to the skin surface, were made from the inner surface with a freezing microtome and stained with 0·5% toluidine blue. The images of the sections were projected on white paper using a field of 2·19 sq.mm. Ten counts were made of the number of hair follicles at a level near the surface of the skin in the various sections, and the results averaged.

As a measure of the milk production of the cows, it was decided to use the milk yields for the first 180 days of lactation, since it is well established that there is a high correlation of around 0·9 between the 180-day yield and the yield for the whole lactation (Tuff (18), Zorn & Funke (19), Johansson & Hansson (20)). The Russian workers whose publications have been cited, e.g. Burcev (14), adopted the 300-day yields, but few of the cows used in the present work had a lactation period extending to 300 days.

The results for herd A are given in Table 7. From the records of the milk yields of the cows the average 180-day milk yields for the first three lactations were obtained. The correlation coefficient between the number of sweat glands in the ear and the milk yield was found to be 0·030 and was not significant. The 180-day yield for the same cows for the period 1946–7 was next taken, corrections being made for the age of the cows, using the age-correction factors of the Dairy Herd Improvement Association as published by Rice (21) and averages for each cow for the 2 years were calculated. The correlation coefficient between

these yields and the number of sweat glands in the ear was 0·072 and again was not significant.

The results for herd B are given in Table 8. The correlation coefficient between the milk yield and the number of sweat glands in the ear was −0·111 and was not significant.

From the very inadequate description which Burcev (14) gives of his methods it is not possible to be sure that his procedure was repeated in every detail. He appeared to make counts on both the inner and outer surfaces of the ear at random and averaged the counts. In an attempt to follow this procedure the experiments which have just been described were repeated with the same skin samples, but counts were made not only at a level near the surface but on both sides of the cartilage in a field area of 20 sq.mm. There is no need to give detailed tables of the results. It will suffice to record that the correlation coefficients obtained in this second experiment for herds A and B corresponding to the three correlation coefficients in Tables 7 and 8 for the first experiment were 0·091, 0·134 and −0·238 and none of them was significant.

Table 7. *The milk yields and number of sweat glands per sq.cm. of skin from the pinna of the right ear of cows from herd A (Exp. 1)*

Cow	Year of birth	Av. 180-day milk yield of the first three lactations (lb.)	Av. 180-day* milk yield of the two lactations in 1946 and 1947 (lb.)	No. of sweat glands per sq.cm. of skin in pinna
Dora	1941	6098	7065	1348
Misty Morn	1940	5382	6325	1238
Jennifer I	1941	5964	5485	1680
Gadfly	1938	4029	5125	1294
Griselda	1940	5694	4581	763
Gertrude	1940	4422	5108	918
Giddy	1943	6976	7645	986
Eve	1943	6113	8565	1308
Effie	1943	6032	9005	1152
Trixie	1943	5754	6805	1165
Dorothy	1943	5516	6870	781
Dinky	1943	5247	5800	1442
Dewdrop II	1943	5229	6140	1341
Joyce	1942	5159	5500	1023

(1) Correlation between the number of sweat glands per sq.cm. of skin and the average 180-day milk yield of the first three lactations:

$r = 0{\cdot}030$, not significant.

(2) Correlation between the number of sweat glands per sq.cm. of skin and the average 180-day milk yield of the two lactations in 1946 and 1947 (with corrections made for age):

$r = 0{\cdot}072$, not significant.

* Corrections made for age.

It appeared therefore from the statistical analysis of the data that the number of sweat glands per unit area of the ear obtained by the methods which have just been described was not an indication of milk-producing capacity in the Ayrshire cow, and it is of interest to observe that in herd A (Table 7) one cow gave an average of nearly 7000 lb. of milk in the first three lactations and had a sweat-gland count of 986 per sq.cm., whereas another cow giving a considerably lower yield of just under 6000 lb. had a count of 1680. Similarly, in herd B (Table 8), the highest yielder (10,994 lb.) had the lowest count (1073) while the lowest yielder (3945 lb.) had a fairly high count (1370).

Experiment 2. In Exp. 1 the object was to repeat Burcev's methods as far as possible,

Table 8. *The milk yields and number of sweat glands in the pinna of the right ear of cows from herd B (Exp. 1)*

Cow no.	Age	180-day yield for one lactation (lb.)	Sweat glands per sq.cm. of skin in the pinna
52	6	5,166	1,290
72	6	6,078	1,174
28	6	8,331	1,174
21	6	8,058	1,506
3	14	8,996	1,476
34	6	8,341	1,700
25	3	4,795	1,484
11	8	10,994	1,073
58	5	4,427	1,223
62	6	4,828	1,415
84	5	3,945	1,370
16	3	4,013	1,141
60	5	4,854	1,732
22	6	8,929	1,200
5	8	9,281	1,392
80	6	5,687	1,302

Correlation between the number of sweat glands and the milk yield:
$r = -0{\cdot}111$, not significant.

and like him to pay no particular attention to obtaining the pieces of ear from precisely the same region for all the cows. It was possible, therefore, that if a region of the ear could be chosen in which the sweat-gland count was reasonably constant, a higher correlation between that count and the milk yield might be obtained than was obtained in Exp. 1. Inspection of Table 2 and Text-fig. 1 suggested that in the region *AC* of the lower margin of the outer surface the sweat-gland counts were relatively close in rows 2–4. This suggested that, provided a piece of skin were taken from the ear in this region of 'relative constancy' and counts were avoided close to the margin, i.e. in row 1, any tendency which might exist for the sweat-gland count and the milk yield to be highly correlated would not be masked by the high sweat-gland counts in the extreme margin of the ear or by other large variations in count which occur from place to place in the ear. This region of 'relative constancy' had an area of about 5 sq.cm. Before proceeding with the experiment, however, it was necessary to establish whether this region exhibited this 'relative constancy' in other ears. Three left ears were therefore obtained from the slaughterhouse and region *AC* was cut out, shaved and direct counts of the hairs made in the rows which in Text-fig. 1 are designated nos. 2–5. The results are shown in Table 9, together with the means and standard error of

Table 9. *The number of sweat glands per sq.cm. in an area of skin measuring approximately 5 sq.cm. which was taken from the outer surface of the left ears of three cows at a position shown by the arrow in Text-fig. 1*

Cow ...	A				B				C			
Row ...	2	3	4	5	2	3	4	5	2	3	4	5
Col. 1	257	202	182	187	380	311	262	247	380	251	207	256
2	242	182	222	197	331	261	286	320	297	272	251	277
3	271	281	242	252	469	301	271	242	282	247	272	251
4	301	227	227	242	306	247	271	232	307	242	292	297
5	276	264	192	232	326	321	286	242	336	276	301	282
Average	233·9				295·6				278·8			
S.E.	±7·7				±12·4				±8·2			

Table 10. *The relation between the sweat-gland count in a carefully chosen area of the ear and the milk yield (Exp. 2)*

Cow no.	Age in years	No. of sweat glands/sq.cm.	Milk yield (lb.) Average of two lactations	Milk yield (lb.) Average of three lactations
10	5	258	6281	—
2	5	280	4984	—
5	5	220	7032	—
28	5	289	4816	—
4	5	251	5705	—
19	5	248	7224	—
11	5	290	4890	—
26	5	201	7220	—
16	6	364	6215	6757
12	6	174	6199	6669
8	6	216	5367	6317
22	6	289	4766	5872
14	6	282	5775	6188
17	6	284	6438	6133
13	6	217	4555	5479
9	6	256	3408	4247
18	6	238	6053	6715
3	7	206	5099	6656
15	7	347	5848	6085
27	7	370	6365	6471
20	8	238	6365	6471
24	8	289	4929	5308
6	8	311	5995	6027
25	8	317	5896	6000
21	8	294	6835	6758
7	9	237	6353	6789
23	9	367	5737	5949
1	11	258	5748	6360

Correlations between sweat-gland count and milk yield

Age group (years)	No. of lactations considered	n	r
All ages	2	28	−0·17
All ages	3	20	−0·03
5	2	8	−0·91**
6	2	9	+0·09
6	3	9	+0·07
8	2	5	−0·20
8	3	5	−0·29

** Highly significant, $P<0·01$.

the means. It will be seen that for these three ears, which were chosen at random, the standard error was much the same in this region. This being so, the following experiment was performed on the Ayrshire herd of the West of Scotland Agricultural College.

Immediately after the evening milking, pieces of skin were cut from the left ears of twenty-eight cows using the technique described on p. 31, but with the following extra precautions. The pieces of ear were always taken from the region *AC* of Text-fig. 1 which was identified each time by reference to the nearest cartilaginous ridge, and the branding tool was used in that region in such a way that the piece cut was certain to include rows 2–4. A streak of paint on the tool made a mark on the skin and ensured that the position of rows 2–4 in the cut piece could be readily identified. The pieces were fixed and the follicle counts made as previously described on pp. 23–24. The results are given in Table 10,

which also shows the average milk yield of the animals for two lactations, and for three lactations where a third one had been completed. The results are arranged according to the age of the cows, which varied from 5 to 11 years. It is clear that none of the correlation coefficients between milk yield and sweat-gland count was significant except for the 5-year-old group where a high *negative* correlation was obtained.

It will be noted that the counts in Table 10 are considerably less than those given in Tables 7 and 8 for Exp. 1. This is due to the fact that in Exp. 1 the pieces of skin were taken near the margin of each ear, whereas in making the counts in Exp. 2 the area for counting was carefully chosen so as to exclude the margin in which, as is shown in Table 2, the counts are particularly high.

CONCLUSION

The main conclusion to be drawn from these experiments is that the close correlation which was found by Burcev and others to exist between the number of sweat glands per unit area of skin in the ear and the milk yield in Red German cattle does not exist in Ayrshire cattle. It is clear from the present results for Ayrshires that the sweat-gland count cannot possibly be used as an indication of milk-producing capacity.

The results of the present work also show that in Ayrshires, the number of sweat glands per unit area in the skin of the ear varies very greatly from one part of the ear to another and even from one field to another in any particular part, whereas Burcev claimed that for the Red German breed any portion of the ear could be used to obtain a reliable count and that the count so obtained would be representative of the ear as a whole. The present investigation shows also that in the Ayrshire breed the ear of the calf has more sweat glands per unit area than that of the fully grown cow. This again is a conclusion which is completely at variance with the claims of Burcev for the Red German breed.

SUMMARY

1. A study has been made of the distribution of the so-called sweat glands in the ears of Ayrshire cattle.

2. The number of sweat glands per unit area of skin in the ear was found to vary greatly from one part of the ear to another and did not appear to be related to the milk yield. It is concluded that it is impossible to use the sweat-gland count in the ear as an indication of the milk-producing capacity of Ayrshire cattle.

3. The number of sweat glands per unit area in the ear of the very young calf was found to be greater than in the fully grown cow, the number being roughly inversely proportional to the surface area of the ear.

4. These findings for Ayrshire cattle are contrary to those published by a number of other workers for cattle of the Red German breed, who claim that any portion of the ear can be used to obtain a sweat-gland count which is closely correlated with milk yield.

The authors are most grateful to Dr P. S. Watts, F.R.C.V.S., for his help in obtaining the samples of skin. They are greatly indebted to Mr J. P. Maule, Director of the Commonwealth Bureau of Animal Breeding and Genetics, and his colleagues for suggesting this investigation and for supplying translations of Russian papers. They are also grateful to Prof. R. C. Garry and his colleagues at Glasgow, to Dr R. A. Robb and Dr J. A. B. Smith for their helpful interest and criticism. One of the authors (S.H.Y.) received a grant from the Agricultural Research Council during the course of the work.

REFERENCES

(1) Liskin (1912). Cited by Stakalic (22).
(2) Nemilov (1915). Cited by Stakalic (22).
(3) Pesciscev (1926). Cited by Stakalic (22).
(4) Cingovatov (1927). Cited by Stakalic (22).
(5) Lisick (1928). Cited by Stakalic (22).
(6) Simic (1928). Cited by Stakalic (22).
(7) Okaladnov (1932). Cited by Stakalic (22).
(8) Podoba (1933). Cited by Stakalic (22).
(9) Nemilov, A. V. (1927). *Trud. Otd. zootehnika, GIOA*, No. 2.
(10) Zamjatin, M. (1929). Cited by Burcev (14).
(11) Konjkov, S. N. (1930). *Izv. kazan. Inst. sel.-Khoz.* (Cited by Burcev (14).)
(12) Podoba (1944). Cited by Burcev (14).
(13) Krasnokutsk, A. I. (1933). *Trud. Leningrad. kombinata Social Zivotnovostva.* (Cited by Burcev (14).)
(14) Burcev, A. V. (1937). *Usp. zooteh. Nauk.* **4** (2), 137.
(15) Findlay, J. D. & Yang, S. H. (1950). *J. Anat., Lond.* (in the Press).
(16) Yamane, J. & Ono, Y. (1936). *Mem. Fac. Sci. Agric. Taihoku*, **19**, 3.
(17) Yang, S. H. (1948). Thesis, University of Edinburgh, June 1948.
(18) Tuff, P. (1931). *Rep. of Proc. Int. Dairy Congr.* p. 151.
(19) Zorn, W. & Funke, A. (1935). *Züchtungskunde*, **16**, 193.
(20) Johansson, I. & Hansson, A. (1940). *K. LandtbrAkad. Handl., Stockh.*, **79** (3), 1.
(21) Rice, Y. A. (1942). *Breeding and Improvement of Farm Animals.* New York: McGraw-Hill.
(22) Stakalic, E. P. (1945). *Izv. Akad. Nauk. S.S.S.R.* (Ser. biol.), p. 722.

(*MS. received for publication* 23 *September* 1949)

619.2:618.19

408. UDDER INFECTIONS IN THE 'DRY PERIOD'. I

By F. K. NEAVE, F. H. DODD and ETTIE HENRIQUES
National Institute for Research in Dairying

(With 5 Figures)

Methods of drying-off cows at the end of each lactation period may have a relatively important influence on the establishment of infections of the udder and on latent infections; this has been pointed out by Munch-Petersen (1) and by Steyn (2). There is still, however, little information about the numbers, types and duration of infections occurring in the drying-off and dry periods and the influence of these infections on milk yield in the subsequent lactation.

In the present study of new infections in the dry period, data have been collected over a period of 3 years in an attested Shorthorn herd (herd N) free from contagious abortion. In the 3 years 1946–8 the average weekly number of cows in milk and the average age of the herd in lactations were 45·4, 49·8, 47·7 and 2·75, 2·48 and 2·34 respectively. The total number of cows infected with *Streptococcus agalactiae* in these years was 16, 11 and 5, with other streptococci 7, 10 and 12, and with staphylococci 6, 19 and 35. From May 1946 clinical cases only were treated with penicillin.

METHODS

When the daily milk yield had dropped to 8–10 lb. the animals were dried off in 1 week by intermittent milking, i.e. by milking the udder out once a day for 1 week only. Once the cows ceased to be milk-recorded no fluid was drawn except for the small quantities of secretion needed for the bacteriological examination.

Fore-milk samples were taken from each quarter 3 days before the final milking-out of the udder, 7, 14 and 21 days later, about 1 week before calving and on the day of calving. Subsequent samples were taken from all quarters of non-infected cows on the 7th and 10th days after calving and from infected cows on the 3rd, 5th, 7th and 10th days. During lactation samples were taken at intervals of 1–2 months or more frequently.

Both during lactation and in the dry period any change in the bacteriological state of the udder was followed by one or more confirmatory samples.

One-ounce samples were taken during lactation, and 1–5 ml. samples during the dry period. From the vast majority of dry cows no difficulty was experienced in obtaining sufficient secretion for bacteriological examination. The few cases of difficulty were caused by the absence of detectable amounts of secretion and not by difficulty in expressing it.

Until May 1946, the herd was milked by hand. Subsequently, part of the herd was milked by a bucket-type milking machine and carefully stripped by machine. From September 1946 the herd was machine-milked, though a few cows were hand-milked throughout 1947.

The hands of milkers were not washed before milking each cow and disinfectants were not used for udder washing throughout the period under review, though dipping the teat

cups in hypochlorite solution was practised with twenty cows in the latter part of 1946 only. Infected animals were not segregated.

Bacteriological methods

Milk samples were taken after careful examination and thorough swabbing of the teat orifice with sodium hypochlorite, followed by drying. About 0·05 ml. of each sample was plated on the surface of aesculin ox-blood agar using two 7 mm. wire loops. Samples from clinical cases were generally plated before and after a 1 : 20 dilution in skim milk.

All colonies were recorded. When colonies of streptococci or staphylococci appeared for the first time or if there was a very mixed flora, indicating careless sampling, additional samples were taken.

An intramammary infection was assumed to be present if the organism was recorded in large numbers on two or more occasions, except that in six cases the presence of the organism in pure culture on a single occasion was accepted as indicative of an infection. The presence of the organism on a number of occasions when sores or abrasions were present at the teat orifice was not accepted as indicative of an intramammary infection unless there were clinical symptoms or distinct increases in the Whiteside and electrical conductivity readings.

Streptococci were identified by the appearance of colonies and by physiological and serological methods. Staphylococci were distinguished from micrococci by colony appearance and by the slide coagulase test with human plasma. Only one or two infections were caused by non-haemolytic staphylococci, and all staphylococcal colonies were pigmented.

Definitions

'*Dry periods*'. For the sake of convenience the term 'dry period' in this paper refers to the period 14 days before the final milking-out of the udder to 14 days after parturition.

True dry period or dry period proper. This refers to the period when the cows were not milked or stripped out (i.e. from the last milking at drying-off until calving).

Persisting infections. This refers to (1) new infections in the 'dry period' which were still present 14 days after parturition, or (2) new infections occurring during lactation and persisting to the end of lactation or until clinical symptoms developed.

Infected on drying-off. This applies to cows found infected before the last 14 days of a lactation. The infection may have been present for several weeks only or for many months.

Free from infections at the beginning of the dry period. This means free from infection 14 days before the final milking-out of the udder.

New infections in cows already infected in one or more quarters. These are new infections occurring in the normal (uninfected) quarters of infected cows.

Clinical. Inflammation, tenderness, distinct clots or discoloured milk, other than 'pink' milk, were regarded as clinical symptoms.

'*Green*' *streptococci.* This is a term used for convenience. It refers to streptococci associated with udder infections which produce green zones or diffuse browning or greening of aesculin blood agar, i.e. *Str. dysgalactiae*, *Str. uberis*, streptococci of groups D and E and unidentified streptococci which split aesculin.

RESULTS

The data have been examined with respect to the number of cows and quarters showing new infections, the type of infection, the number of infections in different stages of the dry period and the number occurring in successive dry periods.

With each of the above methods of examination is recorded the number of infections persisting for at least 14 days after the cow had calved and the proportion of these which showed clinical symptoms during this period.

New infections have been recorded separately for cows which were free from infection in all quarters shortly before becoming dry, and for cows which were already infected in one or more quarters.

A comparison has also been made between the numbers and types of new infections occurring in the true dry period and those occurring in the lactation previous to the dry periods studied.

The high incidence of infections occurring in the dry periods, the number persisting and the number producing clinical symptoms

The number of dry periods included in the investigation was ninety-four; seventy-five of these concerned cows which showed no infection before drying-off and nineteen concerned cows already infected in one or more quarters (Table 1). A few of the cows had two or three dry periods during the investigation.

New infections occurred in 48% of the ninety-four cows and in 24% of the 346 quarters examined. Taking the results as a whole, about half the dry period infections persisted, and about half of the persistent infections became clinical. This was found to be the case for both infected cows and infected quarters.

As might be expected, a significantly higher ($P < 0{\cdot}01$) proportion of new infections (cow and quarters) occurred in cows which were already infected in one or more quarters than in cows free from infection in all quarters before drying-off. There was also some evidence that new infections in cows already infected were more persistent than those in cows previously free from infection ($P = 0{\cdot}05$).

Though the proportion of persisting infections which gave rise to clinical symptoms appeared greater in cows previously free from infection, and old infections appeared to persist longer than new infections in the same cows, these differences were not significant ($P > 0{\cdot}1$).

Of the twenty-four old infections which persisted only three were known to contract new infections, i.e. became mixed infections in the same quarter.

The number of cows infected in lactation and during the dry period

Of the ninety-four cows studied thirty-two developed new infections in the lactations immediately before the dry periods studied, whilst thirty-nine became infected in the true dry period. These infections persisted in twenty-seven of the cows infected during lactation, but in only eighteen of those infected during the dry period. This is more clearly seen in Table 2 where the difference in persistency between new infections in the dry period and those occurring during lactation is statistically significant ($P < 0{\cdot}05$).

Table 1. *New infections in the dry period, the number persisting and the number clinical on calving*

	Cows not infected before drying-off		Cows infected in one or more quarters on drying-off		Totals			Infected quarters of cows infected before drying-off
	Cows	Quarters	Cows	Non-infected quarters	Cows	Non-infected quarters		
Number	75	300	19	46	94	346	—	30
Number becoming infected in one or more quarters in the dry period	30	61	15	21	45	82	Contracting further infections	3
Number persisting in one or more quarters	13/28*†	22/57*†	8	13	21/43*†	35/78*†	Old infections persisting	24
Number clinical at calving	7	13	3	5	10	18	Old infections clinical	10
Proportion becoming infected (%)	40·0	20·3	79·0	45·7	47·9	23·7	Proportion of old infections persisting	80
Proportion persisting (%)	46·5	38·6	53·3	61·9	48·9	44·9	—	—
Proportion of persisting infections clinical at calving (%)	53·8	59·1	37·5	38·5	47·6	51·5	Proportion of old infections clinical	41·7

* Results not included because one cow died after calving.
† Results not included because quarters were treated with penicillin during the dry period.

Table 2. *A comparison of the new-infection rates during lactation and in the dry period proper (eighty-nine cows)*

	Average length of period in weeks	New infections		Infection rate/week	
		Total	Persisting	For all infections	For persisting infections
21 days after the last milking	3	51	21	17	7
The rest of the dry period	11	7	2	0·64	0·18
Total dry period proper	14	58	23	4·14	1·64
Previous lactation	41	63	46	1·54	1·12

Types of infection in the dry periods

Most of the infections in the dry periods were caused by 'green' streptococci and staphylococci, a few were caused by *Str. agalactiae* and a few by coliform organisms (Table 3, Fig. 1).

Of the ninety-one new infections found, eighty-seven were examined over the whole dry period (four could not be examined fully as one cow died on calving and another was treated with penicillin during the dry period). Thirty-seven of the infections persisted to the end of the first 14 days of lactation or longer, and nineteen caused clinical symptoms at calving.

Although forty-three of the ninety-one infections were caused by 'green' streptococci only ten out of forty-two persisted (Table 3), while twenty of the thirty-one staphylococcal

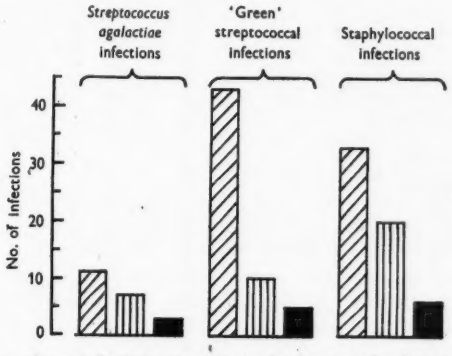


Fig. 1. The types of new infections occurring in the dry periods of ninety-four cows, the number persisting and the number clinical at calving.

▨ new infections; ▥ persisting infections; ■ clinical infections.

infections and seven of the eleven *Str. agalactiae* infections persisted. This difference in persistency between the two former types of infection is highly significant ($P < 0{\cdot}01$). With each type of infection about half of those which persisted gave rise to clinical symptoms in the first 14 days of lactation.

The number of infections of each type present in cows infected before entering the dry period is shown in brackets in Table 3, alongside the new infections in these cows. The ratio of new to old staphylococcal infections was significantly lower than the ratio of new to old streptococcal infections ($P < 0{\cdot}01$).

Str. dysgalactiae was responsible for only four of the forty-three 'green' streptococcal infections.

Types of infections during lactation and dry periods compared

There were forty-six new infections during the lactation period immediately preceding the dry period of the ninety-four cows examined. Thirty-nine of these infections persisted throughout lactation or until treatment, and twenty-two caused clinical symptoms.

An examination of Tables 3 and 4 and Figs. 1 and 2, shows that the total number of new *Str. agalactiae* and staphylococcal infections in both the lactation and dry period was of the same order, while both the number and proportion of 'green' streptococcal infections

Table 3. *Types of new infections occurring in dry period*

	Str. agalactiae infections			'Green' streptococcal infections			Staphylococcal infections			Coliform infections			Total		
	Number	Per-sisting	Clinical	Number	Per-sisting	Clinical	Number	Per-sisting	Clinical	Number	Per-sisting	Clinical	Number	Per-sisting	Clinical
In cows *not* infected before entering dry period	5	2	0	33	6/23	4	24	14/22	9	4	0/3	0	66	22/62	13
In cows already infected on entering dry period	6 (4)*	5	3	10 (4)*	4	1	9 (22)*	6	2	0 (0)*	0	0	25	15	6
Total and percentage (in brackets)	11 (12·1)	7	3	43 (47·2)	10/42†	5	33 (36·3)	20/31‡	11	4 (4·4)	0/3†	0	91	37/87†‡	19
Proportion persisting (%)	—	63·5	—	—	23·8	—	—	64·5	—	—	—	—	—	42·5	—
Proportion of persisting infections becoming clinical (%)	—	—	42·9	—	—	50·0	—	—	55·0	—	—	—	—	—	51·4

* Figures in brackets refer to old infections present before the start of the dry period.
† Results not included because one cow died after calving.
‡ Results not included because quarters were treated with penicillin during the dry period.

Table 4. *Types of new infections occurring in the lactation before the dry periods studied*

	Str. agalactiae			'Green' streptococcal infections			Staphylococcal infections			Total		
	Number	Persisting	Clinical	Number	Persisting	Clinical	Number	Persisting	Clinical	Number	Persisting	Clinical
Total	8 (17·4%)*	8	7	7 (15·2%)*	4	4	31 (67·5%)*	27	11	46	39	22
Proportion persisting (%)	—	100	—	—	57·1	—	—	87·1	—	—	84·8	—
Proportion of persisting infections clinical during lactation (%)	—	—	87·5	—	—	100	—	—	35·5	—	—	56·5

* Proportion of total infections.

was very considerably higher in the dry period, i.e. forty-three compared with seven during lactation, or 47% of the total infections compared with 15%.

The differences in persistency between new infections in the dry period and during lactation, mentioned above, appear to be true for the three types of infection (cf. Figs. 1 and 2).

The total *number* of new infections which persisted and the number which caused clinical symptoms were surprisingly similar in the two periods. Thirty-nine persisted and twenty-two gave rise to clinical symptoms during lactation, while thirty-seven persisted and nineteen caused clinical symptoms in the subsequent dry period. Thus, of the combined total of forty-one clinical cases found, nineteen occurred in the 14 days after the animals had calved.

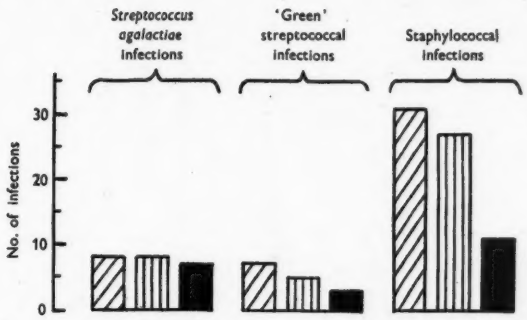


Fig. 2. The types of new infections in the ninety-four lactations prior to the dry periods studied, the number persisting and the number clinical.

Key as in fig. 1.

That a high proportion of clinical cases of mastitis occurs very early in lactation has, of course, been observed by many farmers.

It is of some significance that of the forty-one clinical cases found only ten were caused by *Str. agalactiae*, while twenty-two were caused by coagulase-positive staphylococci, and also that as infections caused by *Str. agalactiae* decreased those caused by other organisms increased.

Infections found in successive stages of the dry periods

It is of interest to determine at which stage of the dry period most new infections develop. From our data it was possible to determine when seventy-six of the ninety-one dry-period infections started. These details are presented in Table 5 and Fig. 3, where the number of infections which were found in the drying-off period, i.e. in the 14 days before the final milking-out of the udder, the number found in the next 21 days, in the remainder of the dry period proper and in the 14 days after calving, are recorded.

Ten infections occurred in the first period, fifty-one in the second, seven in the third and eight in the last. The fifty-one infections in the second period comprised six attributed to *Str. agalactiae*, twenty-four to 'green' streptococci and twenty-one to staphylococci; and of the eight infections of the last period two were caused by *Str. agalactiae*, three by 'green' streptococci and three by staphylococci.

Undoubtedly the first few weeks of the dry period proper was the stage when the majority of infections were found. More frequent sampling would, no doubt, have led to a more precise statement and might also have altered slightly the proportions recorded,

Table 5. *New infections found in successive stages of the dry period*

	In the 14 days before the final milking-out			In the 21 days after the final milking			In the remainder of the dry period			In the 14 days after calving			Total		
	Number	Persisting	Clinical	Number	Persisting	Clinical	Number	Persisting	Clinica	Number	Persisting	Clinical	Number	Persisting	Clinical
Infections in cows *not* infected before entering dry period	9	2	1	32	10	6	6	1	0	4	3	2	51	16	9
Infections in cows already infected at start of dry period	1	1	0	19	11	4	1	1	1	4	2	1	25	15	6
Total and percentage (in brackets)	10 (13·2)	3	1	51 (67·1)	21/49*†	10	7 (9·2)	2/5†	1	8 (10·5)	5	3	76	31/72*†	15
Proportion of infections persisting (%)	—	30·0	—	—	42·9	—	—	40	—	—	62·5	—	—	43·1	—
Proportion of persisting infections clinical (%)	—	—	33·3	—	—	47·6	—	—	50	—	—	60	—	—	48·4

* Results not included because one cow died after calving.
† Results not included because quarters were treated with penicillin during the dry period.

Table 6. *New infections occurring in successive dry periods*

	First dry period			Second dry period			Subsequent dry periods			All dry periods		
	Number	Persisting	Clinical	Number	Persisting	Clinical	Number	Persisting	Clinical	Number	Persisting	Clinical
Total number of dry periods	31	—	—	24	—	—	39	—	—	94	—	—
Dry periods in which new infections occurred	7	2	1	13	5	3	25	14/23*†	6	45	21/43*†	10
Proportion of dry periods with new infections (%)	22·6	—	—	54·2	—	—	64·1	—	—	47·9	—	—
Proportion of new infections which persisted (%)	—	28·6	—	—	38·5	—	—	60·9	—	—	48·9	—
Proportion of persisting infections which became clinical (%)	—	—	50·0	—	—	60·0	—	—	42·9	—	—	47·6

* Results not included because one cow died after calving.
† Results not included because quarters were treated with penicillin during the dry period.

but not, we think, sufficiently to change the picture in Table 5. The relatively high proportion of infections which were found in the second period, i.e. in the first 3 weeks after the cow was officially dry applied to cows already infected as well as to cows free from infection before entering the dry period.

Fifty-eight of the new infections were first found in the dry period proper; thirty (52%) of these disappeared before the cows calved and would therefore not have been discovered had bacteriological tests not been made during the dry period.

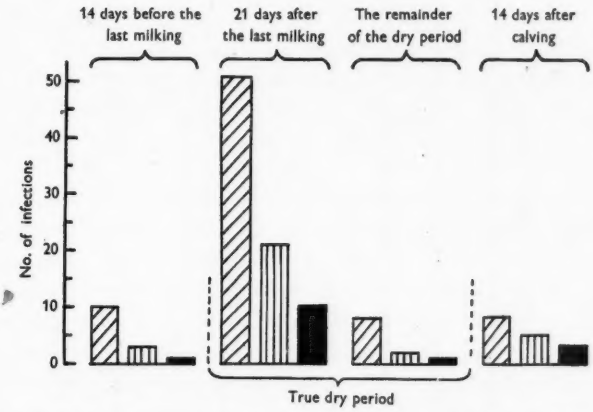


Fig. 3. Infections found in successive stages of the dry period (seventy-six cows).
Key as in fig. 1.

The rate of new infections

In order to emphasize the relatively large number of infections starting in the dry period, particularly in the early dry period, the average new infection rates per week have been estimated and are shown in Table 2 and Fig. 4. The most significant information in the table is the high average new infection rate, 17 per week, during the first 21 days of the dry period compared with 4·14 per week for the whole of the dry period and 1·54 per week during the previous lactation.

Of more practical significance perhaps, because of the effect on milk yield, was the high average rate of new infections per week which *persisted*. This was very significantly greater in the early dry period than in any other period studied, but was not significantly greater in the dry period as a whole than in the previous lactation.

New infections occurring in successive dry periods

It is generally known that a higher proportion of older animals are found infected than younger animals, that the former have also a larger number of infected quarters, and although the reasons may not be entirely clear there are now considerable published data indicating that the oldest animals are usually the most susceptible to new infections. This is true for *Str. agalactiae* infections and possibly true for staphylococcal infections.

In Table 6 and Fig. 5 the number of infections occurring in the first, second and subsequent dry periods is shown. The figures indicate that the number of infections in dry

periods is probably related to age of animal ($P < 0{\cdot}05$). The proportion of infections which persisted in the different dry periods appears to be similarly related to age, though the figures shown are not statistically significant.

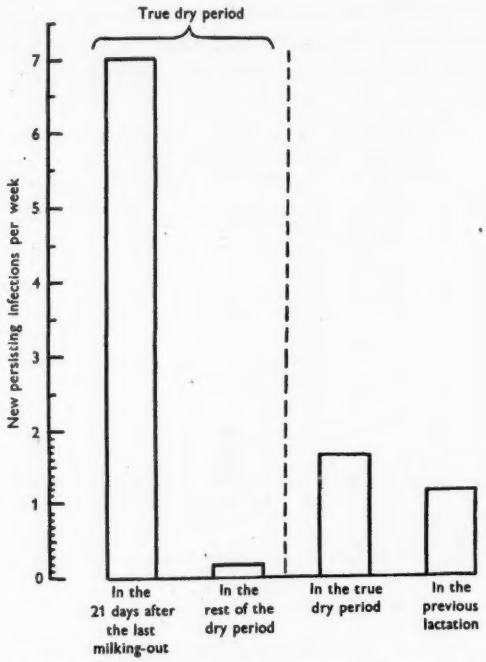


Fig. 4. The rate at which new persisting infections appeared during lactation and in the dry period proper.

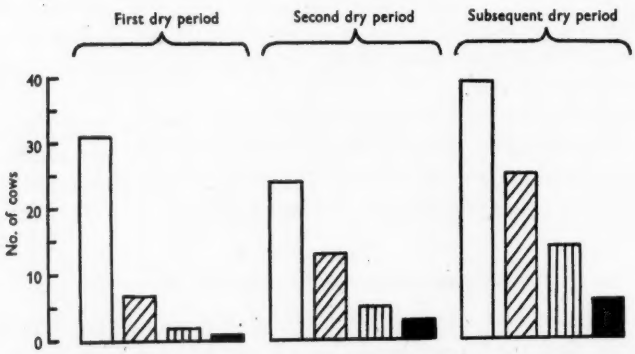


Fig. 5. The relation between age and dry period infections.

□ cows examined; ▨ cows infected in dry-period; ▥ cows with persisting infections; ■ cows clinical at calving.

DISCUSSION

Our evidence seems to show that in the herd examined new infections were present in about half the dry periods investigated (i.e. in the period 14 days before the final milking-out of the udder to 14 days after calving), that half of these infections persisted for at least the first 2 weeks of lactation and that, of the latter, 50% gave rise to clinical symptoms shortly after calving.

It has likewise been shown that in this herd, which was known to have a decreasing incidence of *Str. agalactiae* infections, about half the new dry-period infections were caused by 'green' streptococci (forty-three out of ninety-one), but that most of the infections which persisted (twenty out of thirty-seven) were caused by staphylococci, while of the nineteen quarters showing clinical symptoms during the first 2 weeks of lactation, eleven were infected with staphylococci, five with 'green' streptococci and three with *Str. agalactiae.*

There was evidence of an increase in the number of dry-period infections with age of cow, and also in cows already infected in one or more quarters, compared with cows free from infection in all quarters, before being dried off.

Of the eighty-seven infections fully examined, fifty did not persist. Thirty-six of the spontaneous recoveries occurred before the cows calved and fourteen after calving but within the first 14 days of lactation. A few of the dry-period infections which disappeared before the cows calved affected the yield of the quarter, while a proportion of those which disappeared spontaneously within the first 14 days of lactation had a marked effect on yield. We hope shortly to publish a fuller report of these infections.

More than half the infections were found within the first 3 weeks of the dry period proper, i.e. after the final milking-out of the udder. The inference from this is either that the method of drying-off cows may have considerable influence on the amount of infection at this period, or that sampling itself predisposes the udder to infection (either by the removal of 1–5 ml. of secretion or by opening the orifice), or that the udder is more susceptible to infection at this period. These questions are now being studied more fully.

In none of the 3 years were 'green' streptococcal infections found to predominate in cows examined during lactation. The fact that these organisms were the predominant cause of the infections which started in the early dry period and disappeared spontaneously in the late dry period or early lactation is probably connected with the marked changes in the internal environment of the udder occurring during these periods.

Although there was a marked increase in the total annual number of infected cows found in the period under review there was no increase in the ratio of persistent infections which started in the dry period proper, to those occurring during lactation.

There does not appear to be agreement as to the stage of lactation or dry period in which most infections begin. Seelemann's (3) data show that the seventy new infections were spaced fairly evenly throughout the lactation including the dry period. Kästli (4) examined the distribution of infections in over 1000 cows, and whereas it is not possible to determine whether or not he was dealing with new infections, slightly more infected cows appear to have been found in early and late lactation. Ineson & Cunningham (5), in agreement with some of the earlier investigators (Munch-Petersen (6)), found most new infections at the first test after calving though not significantly more than at other periods. Stylianopoulos (7) recorded the distribution of 117 clinical cases; twenty-eight were observed in the first

14 days of lactation and thirty-one in the drying-off and dry periods. Arthur (8), in a similar investigation of 395 clinical cases, found 38% in animals being dried off or before they calved.

Unless samples of secretion are taken at the end of a lactation period, before and immediately after calving, it is not possible to distinguish between new infections occurring in late lactation and those starting in early lactation. The observations recorded in this paper show that new infections occurred at a much higher rate during the dry period ($P < 0{\cdot}01$), particularly the early part of the dry period, than in the previous lactation and that the total number of infections was greater in the dry period than in the previous lactation.

The safest method of drying-off cows has long been debated. Wayne, Eckles & Petersen (9) and Wayne & Macy (10), as a result of their experiments, favour abrupt cessation of milking, as also does Steyn (2) in his review. Johnson (11) advises intermittent milking, which appears to be the method most frequently used in practice, though some farmers apparently practise incomplete milking.

There would appear to be little reliable information concerning the effect of the different methods of drying off the udder on the course of latent infections, on the incidence of udder infections and the influence of these infections on milk yield in the subsequent lactation. An examination of the data of Wayne *et al.* (9), however, shows that the right half of the udder of cows 138 and 536 (their Table 4), which was dried off by intermittent milking, exhibited a drop in milk yield in the following lactation which could be attributed to a dry-period infection. None of the eight cows showed a drop in the yield of the halves of the udder dried off by complete cessation of milking.

The influence on mastitis of the milking methods at parturition has received even less attention from research workers than the method of drying off. Keyes & Reid (12) have reported that many streptococcal udder infections disappear if the cows are 'prepartum milked'. It would be interesting to know whether these are the normal spontaneous recoveries of the late dry period or whether there is, in fact, a greater reduction in the number of infections and clinical cases found at calving.

There does not appear to be any information about the relative anti-bacterial properties of secretions of the gland at various stages of lactation and dry periods nor very much information about the changes in secretory tissue during a *normal* dry period.

SUMMARY

In a herd averaging forty-five to fifty cows in milk, large numbers of infections occurred during the dry period and over half of these developed during the first 3 weeks after the final milking-out of the udder. About 50% of the infections persisted and a like percentage of these produced clinical symptoms within the first 2 weeks of calving, half of them caused by staphylococci.

We wish to thank Dr A. T. R. Mattick and Mr A. S. Foot for their support and interest.

REFERENCES

(1) Munch-Petersen, E. (1938). *Rev. Ser. Imp. Bur. anim. Hlth*, **1**, 61.
(2) Steyn, H. P. (1940). *J. S. Afr. vet. Med. Ass.* **11**, 123.
(3) Seelemann, H. (1932). *Die Streptokokkeninfektionen des Euters*, p. 36. Hannover: Schaper.
(4) Kästli, P. (1933). *Schweiz. Arch. Tierheilk.* **75**, 461.
(5) Ineson, P. June & Cunningham, A. (1949). *J. Dairy Res.* **16**, 139.
(6) Munch-Petersen, E. (1938). *Rev. Ser. Imp. Bur. anim. Hlth*, **1**, 13.
(7) Stylianopoulos, M. (1934). *N. Amer. Vet.* **15**, 22.
(8) Arthur, G. H. (1947). *Vet. Rec.* **59**, 231.
(9) Wayne, R., Eckles, C. H. & Petersen, W. E. (1933). *J. Dairy Sci.* **16**, 69.
(10) Wayne, R. & Macy, H. (1933). *J. Dairy Sci.* **16**, 79.
(11) Johnson, S. D. (1941). *Cornell Vet.* **31**, 127.
(12) Keyes, E. A. & Reid, J. J. (1945). *Bull. Pa agric. Exp. Sta.* no. 475, p. 14.

(*MS. received for publication* 4 *October* 1949)

Addendum

Since going to press, A. D. McEwen and Marjory White (*Vet. Rec.* 1950, **62**, 27) in an interesting paper have shown that the bactericidal and bacteriostatic titres of milk fall during the drying-off period when they may reach a low level.

637.125:614.48

409. APPARATUS FOR STUDIES ON DISINFECTION OF MILKING MACHINE TEAT CUPS

By G. E. K. LINEHAM

Boots Pure Drug Co. Ltd., Research Department, Veterinary Science Division, Thurgarton, Notts

(With 3 Plates and 2 Text-figures)

Any measures designed to control the spread of chronic contagious mastitis in cows caused by *Streptococcus agalactiae* should include some efficient method of sterilizing the teat cups of milking machines after the milking of each cow.

In the course of research at these laboratories into the factors involved in the control of spread of this infection it became necessary to study the efficiency of the recommended procedures for sterilizing teat cups and their possible improvement.

Mallmann & Leavitt (1) have repeatedly emphasized the necessity for testing disinfectants under conditions resembling those under which they are normally used and by actual field trials while in use. However, there are considerable difficulties in producing comparable results under conditions in the cow-shed. The excretion of *Str. agalactiae* from an infected quarter varies widely from one milking to another, the character of the secretion changes and these differences may be appreciable between different cows or even different quarters of the same cow.

It became obvious that a procedure would have to be evolved which would allow equal degrees of contamination of different teat cups which could then be treated comparatively. This article describes two pieces of apparatus which facilitate such comparative studies.

APPARATUS FOR THE COLLECTION OF TOTAL YIELDS OF EACH QUARTER OF THE UDDER

Before the efficacy of disinfection can be assessed, it is desirable to know the total number of streptococci passing through a particular teat cup. This figure can then be compared with the number of organisms surviving any disinfectant procedure applied subsequently. For this purpose it is necessary to be able to collect the individual yield of each quarter of the udder. Specially designed milking machines for the collection of individual quarter yields have been described by McDowall (2), but the following adaptation of a standard type milking machine is more easily and cheaply made.

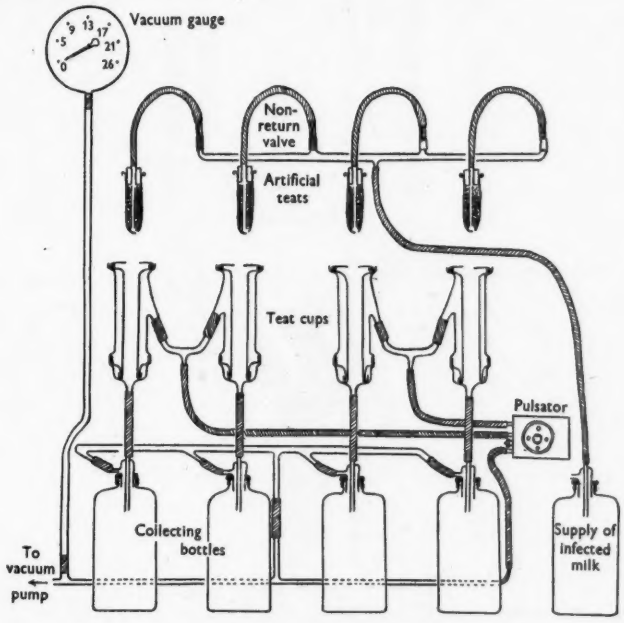
Description of apparatus

A milking machine twin pulsator tube was attached at one end to two pieces of glass tubing passing through a rubber bung into a Winchester quart bottle and at the other end to two pieces of glass tubing bent as shown in Pl. 1. These were passed through a rubber bung for support and the free ends fitted with small pieces of strong rubber tubing. The usual rubber tubing forming the connexion from the bottom of the teat cup to the claw (i.e. the short milk tube) was removed and replaced by the above attachment. Similar attachments were prepared for the other teat cups. The vacuum pipes between each teat cup and the claw were left undisturbed. When milking commenced the milk from each teat fell directly into the individual Winchester quart bottle instead of passing through the claw and long milk tube to the milking pail. The milking pail was left in position and acted only as an air reservoir.

LINEHAM—Apparatus for studies on disinfection of milking machine teat cups

Points to remember when preparing the apparatus are first, that all connexions must be airtight since the apparatus works at a reduced pressure, and second, that the glass tube taking the milk into the Winchester must be longer than the air return tube in the same bung or some milk will be drawn into this latter tube. Pl. 1 shows the apparatus in use in the cow-shed. After any test, the attachments may be quickly removed and the machine is available for normal milking simply by replacing the short milk tubes.

Using the above apparatus, the complete yield from an infected quarter can be collected for examination in the laboratory. The number of organisms passing through the teat cup may be estimated and related to the number of organisms on the teat cup liner after treatment with disinfectant.



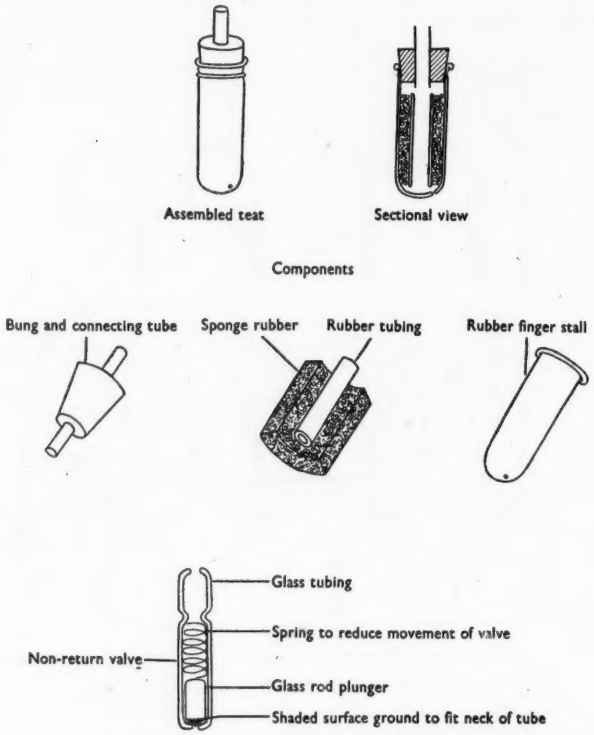
Text-fig. 1. Apparatus for artificially infecting teat cups.

Although the collection of individual quarter yields greatly aided our studies on teat-cup disinfection, a number of disadvantages continued to hamper the work: (1) The sampling of the teat-cup liners before and after treatment had to be done in the cow-shed with the attendant risks of contamination by air-borne organisms. (2) At any one milking, it was not possible to evaluate on one teat-cup liner both the number of organisms retained after milking and the number surviving disinfection, since the first procedure would involve the mechanical removal of the majority of the deposited organisms. (3) Only one disinfection test a day could be performed with any one infected cow.

EXPERIMENTAL MILKING APPARATUS FOR LABORATORY USE

To obviate these disadvantages an attempt was made to reproduce as closely as possible, in the laboratory, the normal conditions of machine milking by the use of a form of artificial udder. This technique of milking from an artificial udder has been recorded

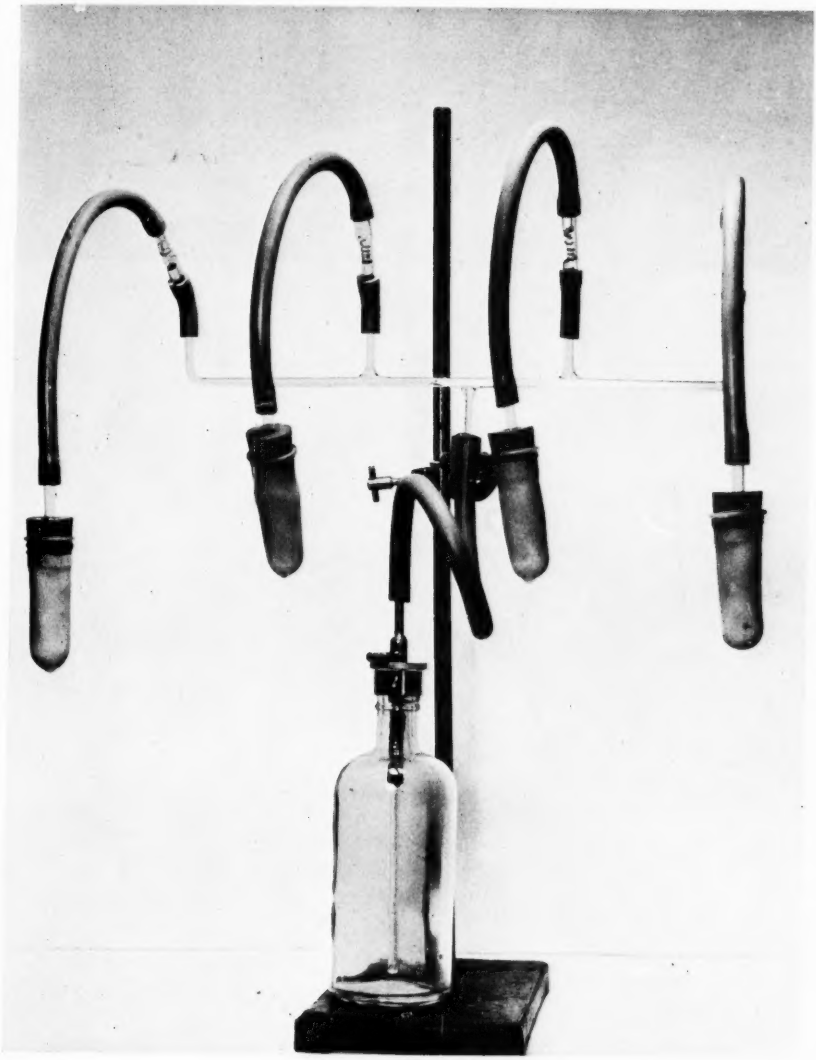
previously, but it has not been used as a means of infecting teat cups. It was described originally by Ruehle, Breed & Smith (3) and again by Parfitt (4) as a means of testing the contamination of milking machines. They 'milked' sterile skimmed milk from an artificial udder through the normal milking machine and estimated the number of bacteria in the skimmed milk collected in the bucket. For the purpose of investigations on *Str. agalactiae* the total yield from the infected quarter, collected as described above, was milked through a series of teat cups. The artificial udder took the form of a reservoir feeding four artificial teats. This and the whole apparatus are illustrated in Pl. 2 and 3.



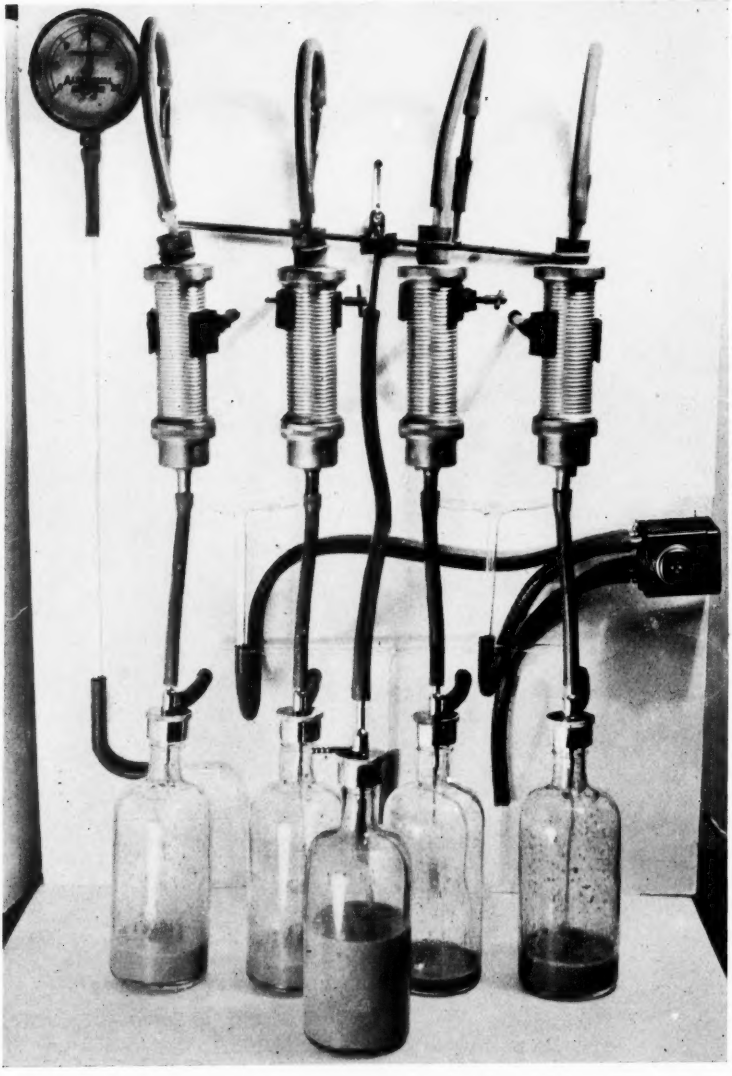
Text-fig. 2. Details of teat and non-return valve.

Description of apparatus

(*a*) *Artificial teats.* These consisted of finger stalls of a size comparable with a natural teat. A small teat orifice was produced by piercing the tip of the finger stall with a hot needle. Care was taken to make this hole small and of the same diameter for each teat, although differences in size of hole can be adjusted by the inclusion of a small piece of standard-bore glass capillary tubing in the rubber tubing immediately above the teat. The finger stall was given a certain amount of rigidity by packing with sponge rubber, wrapped around a small-bore rubber tube, which maintained a regular flow of milk and avoided any filtering off of clots. The assembly was completed with a rubber bung and supply tube and the finger stall kept in place by a rubber band. Milk was supplied from a reservoir through



LINEHAM—APPARATUS FOR STUDIES ON DISINFECTION OF MILKING MACHINE TEAT CUPS



LINEHAM—Apparatus for studies on disinfection of milking machine teat cups

a four-way T-piece, and a non-return valve was provided for each teat. Details of this part of the apparatus are illustrated in Pl. 2 and Text-fig. 2.

(*b*) *Milking machine.* This was composed of four normal teat cups arranged side by side below the teats and activated by the normal pulsator. The milk was collected in individual bottles immediately below the cups and suction maintained through a four-way T-piece; both this and the pulsator were connected to a vacuum pump and a vacuum gauge was included to check that the vacuum employed was that recommended for the particular make of milking machine from which the teat cups and pulsator were taken.

Details of the complete apparatus are illustrated diagrammatically in Text-fig. 1 and in action in Pl. 3. Filtering heads* were used on all bottles to facilitate rapid changing.

Experimental procedure

The whole apparatus was sterilized in free steam at 100° C. before it was fitted up. The infected milk was poured into the reservoir supplying the teats which were then lowered into the teat cups. Vacuum was applied and the milk 'milked' through into the collecting bottles. If the rate of flow differed slightly from teat to teat, it was adjusted by applying a screw clip to the rubber tubing immediately above the teat itself. After all the milk had passed through the teats the vacuum was cut off, the collecting bottles disconnected and the milk poured back into the reservoir. The whole process could be repeated until each cup had received a volume equal to the total yield of the infected quarter. With four cups infected to the same extent it was possible to use one as a control for the weight of infection and the other three were treated with disinfectants for varying times and by various methods. The results obtained using this apparatus will be published in due course.

SUMMARY

Two pieces of apparatus are described for use in studies on disinfection of milking machine teat cups: (1) an easily constructed attachment for a standard milking machine allowing the collection of the total yield from each individual quarter; and (2) an apparatus designed to reproduce as closely as possible machine milking and to allow an equal quantity of the same sample of infected milk to be 'milked' through each of four teat cups which will be infected to the same extent. These infected teat cups can be used to provide comparable results in trials on different disinfection procedures.

Thanks are due to Sir Jack Drummond, F.R.S., Director of Research, for permission to publish this article; to D. L. Hughes, M.R.C.V.S., Dip.Bact. (Lond.), Head of the Veterinary Science Division, for his help and criticism, and to Mr G. Whiting for photographing the apparatus.

REFERENCES

(1) MALLMANN, W. L. & LEAVITT, A. H. (1948). *Amer. J. vet. Res.* **9**, 104.
(2) MCDOWALL, F. H. (1945). *J. Dairy Sci.* **14**, 231.
(3) RUEHLE, G. L. A., BREED, R. S. & SMITH, G. A. (1918). *Bull. N.Y. agric. Exp. Sta.* no. 450.
(4) PARFITT, E. H. (1931). *Bull. Purdue Univ. agric. Exp. Sta.* no. 348.

* Supplied by Messrs Baird and Tatlock.

(*MS. received for publication* 1 *November* 1949)

637.0:612.392.01

410. THE OXIDATION OF ASCORBIC ACID IN DAIRY PRODUCTS

By J. E. ALLAN
Dairy Division's Laboratory, Wallaceville, New Zealand Department of Agriculture

(With 1 Figure)

INTRODUCTION

Although the ascorbic acid content of dairy products is hardly significant from a nutritional point of view, it is important in connexion with the oxidative changes which occur and which lead eventually to flavour defects. The exact role played by ascorbic acid is, however, by no means certain. Some investigators (see Brown & Thurston (1) for a review) state that flavour defects due to oxidative changes do not occur until all the ascorbic acid is oxidized, and that the development of an oxidized flavour is inhibited by the addition of ascorbic acid. Other workers find no close correlation between the amount of ascorbic acid present, or its rate of loss, and the development of flavour defects (2,3). Others attribute to ascorbic acid a part in the production of an oxidized flavour (4) and find that if the ascorbic acid is quickly and completely oxidized no flavour defect develops (5).

Little work appears to have been done on the ascorbic acid content of butter. One author (6) states that it disappears shortly after manufacture. It was considered that this subject was worthy of further investigation as, in spite of the conflict of evidence regarding the part played by ascorbic acid in the development of flavour defects, there is general agreement that it appears to be the first substance to be oxidized in milk. A study of the ascorbic acid content of butter and its rate of disappearance might therefore indicate to what extent oxidative changes had taken place and provide some knowledge of the various factors involved in oxidative changes.

Preliminary work was undertaken with the intention of investigating the vitamin C status of cream, buttermilk and butter, and to that end various analytical methods were investigated. As none proved suitable for butter samples, a different approach to the problem was made and the factors involved in the oxidation of ascorbic acid were studied in a series of kinetic experiments on butter serum, to which ascorbic acid was added, after the separation of the fat.

EXPERIMENTAL

The following is a brief account of the analytical methods investigated with the object of determining the ascorbic acid content of butter.

Oxidation-reduction method. Stewart & Sharp's method (7) in which cucumber juice, containing ascorbic acid oxidase, and a suspension of *Escherichia coli* are used in order to eliminate interfering substances and to determine dehydroascorbic acid as well as ascorbic acid, was tried.

The strain of *E. coli* used was kindly supplied by Dr P. F. Sharp, Cornell University, New York. The cucumber juice was obtained from cucumbers purchased locally; however, not all cucumbers had a sufficient concentration of the enzyme to be of any use. The juice

was extracted as described by Sharp, Hand & Guthrie (8) but was not concentrated. Instead, an appropriately larger amount of the original juice was used.

In this method the sample to be analysed is acidified with sulphuric acid and the ascorbic acid titrated directly with 2:6-dichloro-phenol-indophenol. This procedure was found to be not quite satisfactory, as the end-point was difficult to determine, especially in the case of heated milk, containing interfering substances which caused fading of the end-point colour.

A method involving precipitation of the proteins and filtering was therefore adopted. For this purpose 10% meta-phosphoric acid, as used by Perrin & Perrin (9), was found satisfactory for milk and buttermilk samples. For cream and butter serum samples, however, a mixture of equal volumes of 8% trichloracetic acid and 10% sodium meta-phosphate (10) gave quicker filtering and was therefore preferred.

A buffer, which consisted of 6·13 g. of KH_2PO_4 and 0·89 g. of Na_2HPO_4, $2H_2O$ made up to 100 ml. with distilled water, was used to adjust the pH of the samples to approximately 5·9 as required by the *E. coli*.

The solution of sodium 2:6-dichloro-phenol-indophenol, containing 0·1 g./l., was standardized daily by titration against 5 ml. of a solution of Mohr's salt (0·500 g./l.) as described by Stewart & Sharp (7). The dye factor then equals 0·562 divided by the number of ml. of the dye used in the titration.

For each analysis four titrations were done, each upon 10 ml. of filtrate, obtained by adding 10 ml. of precipitating reagent to four 10 ml. samples after the following treatments:

A. 2 ml. of water were added.

B. 1 ml. of buffer and 1 ml. of *E. coli* suspension were added and the samples incubated for 25–30 min. at 37° C.

C. 1 ml. of buffer, 1 ml. of water and 0·1 ml. of cucumber juice were added, and the samples left at room temperature for 15 min.

D. 1 ml. of buffer, 0·1 ml. of cucumber juice and, after 15 min., 1 ml. of *E. coli* suspension were added. The sample was then incubated at 37° C. for 25–30 min.

The results were calculated as follows:

Dehydroascorbic acid $= [(B-A) \times (22-P) \times \text{dye factor}]$ mg./100 ml.,

Total vitamin C $= [(D-C) \times (22-P) \times \text{dye factor}]$ mg./100 ml.,

where P = volume of precipitate from 10 ml. samples. For milk this would be approximately 0·7 ml., for buttermilk and butter serum 0·4 ml. and for cream (40% fat) approximately 4·5 ml.

When the precipitating reagents mentioned above were used, the interfering substances $(B-D)$ were found to be filtered out with the protein precipitate (see also Doan & Josephson (11)) so that the value of $B-D$ was rarely greater than the experimental error. Thus for most purposes the use of cucumber juice could be omitted and only titrations *A* and *B* done. It was then necessary to subtract a blank value, which was determined by the use of cucumber juice (titration *C* above), copper sulphate, or sunlight. For milk, buttermilk and butter serum a value of 0·15 mg./100 ml. was obtained, and for cream, a value of 0·13 mg./100 ml.

Several attempts to apply the above method to butter were not successful. In order to obtain the serum it was necessary to melt the butter, centrifuge and remove the fat. The above method required that about 200 g. of butter be melted, and using centrifuge tubes

which held 80–90 g. of butter, this took 35–45 min. at a temperature of 45–48° C. Recovery experiments showed that during this time the butter lost 50–80% of its ascorbic acid.

Polarographic method. With a view to doing analyses on a much smaller amount of serum and so reducing the time required for melting the butter, the polarographic method of Perrin & Perrin (*loc. cit.*) for the determination of ascorbic acid in milk was investigated. The apparatus used was very similar, except that the position of the polarity reversing switch was such that the galvanometer was connected to one electrode permanently. The switch then reversed the voltage across the cell and galvanometer together so that the direction of the galvanometer deflexion was not changed unless the direction of the current through the cell changed.

Various difficulties were encountered in the application of the method and it was not further used. In the first place, interference from the high chloride concentration in the serum of salted butter could not be overcome; and secondly, anomalous results were obtained when copper sulphate was used to destroy the ascorbic acid.

Micro-titration method. A further attempt to analyse butter directly for ascorbic acid was made by means of a micro-titration method, needing only 0·5 ml. of butter serum. This was obtained by melting and centrifuging about 5 g. of butter, and was pipetted into 1 ml. of 10% meta-phosphoric acid in a 15 ml. conical centrifuge tube. After centrifuging at 3000 r.p.m. for 15–20 min., 0·75 ml. of the clear layer was withdrawn and titrated.

The micro-burette used was similar to that described by Farmer & Abt (12), and was constructed from a piece of capillary tubing of uniform bore having an internal diameter of slightly less than 1 mm. (so that 0·1 ml. was approximately 18 cm.). The dye solution at a concentration of 0·135 g./l. was standardized by titrating 0·05 ml. of Mohr's salt solution (see above) in 0·1 ml. of meta-phosphoric acid, the Mohr's salt being measured by means of a graduated 0·1 ml. pipette. For the titration of the samples, which was done in a small white porcelain basin, the dye solution was diluted with an equal volume of distilled water.

In order to test the method for butter, about 100 g. of cream were churned by shaking in a bottle. The resulting butter (unwashed and unsalted) was immediately analysed as described above, and the result compared with the analysis of the buttermilk done similarly. When using cream uncontaminated with copper, about 90% of the ascorbic acid in the buttermilk could be found in the butter serum. This was a marked improvement upon previous results, but the addition of a small amount of copper (0·25 p.p.m. to the cream), as often occurs in the manufacturing processes, caused the recovery to drop to 60–70%.

Little improvement seemed possible by reducing still further the amount of serum analysed, for although the melting time (about 5–7 min.) might be reduced, the samples would have to be kept warm during centrifuging and until the fat was removed, and the time required for this would not be reduced by working with a smaller sample.

Kinetic experiments. Owing to the rapid loss of ascorbic acid when the butter was warmed, reliable direct estimation of this substance in butter appeared to be scarcely possible, even with the micro method. The problem was therefore approached in another way by carrying out kinetic experiments upon the rate of change of the ascorbic acid content of butter serum and buttermilk. The micro method of analysis described above was used on account of its advantages over the macro method for this work, both in saving time and in reducing the size of sample required.

For each set of experiments an appropriate amount of cream (1–2 pints) was churned in a glass churn. The buttermilk was drained off and the butter, which was not washed nor salted, was melted and centrifuged to obtain the serum, precautions being taken throughout to avoid metallic contamination. Any ascorbic acid remaining was destroyed by exposure to light. Kinetic experiments were then carried out by adding ascorbic acid and following its disappearance under different conditions.

For each run the reaction mixture contained 5 ml. of serum or buttermilk plus ascorbic acid solution and such other components as were required for the particular experiment, with a total volume of 6 ml. Everything except the ascorbic acid was placed in a test-tube in the thermostat. When the required temperature was reached the ascorbic acid solution was added, mixed, and duplicate aliquots of 0·5 ml. immediately withdrawn and each pipetted into 1 ml. of 10% HPO_3, which served to stop the reaction and precipitate the protein. These were titrated as described above to give the initial concentration of ascorbic acid. Further aliquots of 0·5 ml. were withdrawn at suitable times and analysed. Blank titrations were done on butter serum and buttermilk before the addition of the ascorbic acid solution, and precautions were again taken throughout the procedure to avoid metallic contamination.

All the reactions were carried out in the dark. The concentration of ascorbic acid in the reaction mixture was between 1·4 and 1·8 mg./100 ml. and with this concentration of ascorbic acid the amount of dissolved oxygen in the reaction mixture was sufficient to provide a large excess over that required. All the experiments were done at the pH of fresh cream, i.e. 6·6–6·7. The serum and buttermilk were analysed for copper and iron in duplicate or triplicate by a wet-ashing method (13).

The rate coefficient, k_1, was calculated for each experimental point using the following formula:

$$k_1 = \frac{2{\cdot}303}{t} \log \frac{a}{a-x},$$

where a equals the initial titration, and x the titration at time t, which was expressed in minutes.

Although the k_1 values showed no definite tendency to rise or fall during the course of the reaction, considerable variation was sometimes obtained, owing to experimental errors. These were thought to be chiefly due to (*a*) variable drainage of the pipette, especially when removing the 0·5 ml. aliquot of serum for analysis, and (*b*) the difficulty of observing the end-point in the titration for ascorbic acid, especially in variable light conditions. Towards the end of the work the titrations were carried out with indirect light from a fluorescent 'daylight' lamp which enabled the pink colour of the end-point to be detected much more readily and consistently.

RESULTS

Preliminary investigation established that the oxidation reaction showed first-order kinetics, and that the rate was greater in butter serum than in the corresponding buttermilk. Further, the rate was increased by the addition of cupric and ferric salts, and was decreased by the addition of sodium chloride. The effects of these factors have been studied more fully. A typical set of percentage oxidation/time curves is shown in Fig. 1 and the results are considered in detail below.

The effect of added copper. In Table 1 are shown the k_1 values for three different serums

with and without added copper. The difference between the value for the serum to which no copper was added and the value for the serum plus copper, gives the k_1 value for the added copper, and from this has been calculated the k_1 value for 0·1 p.p.m. added copper.

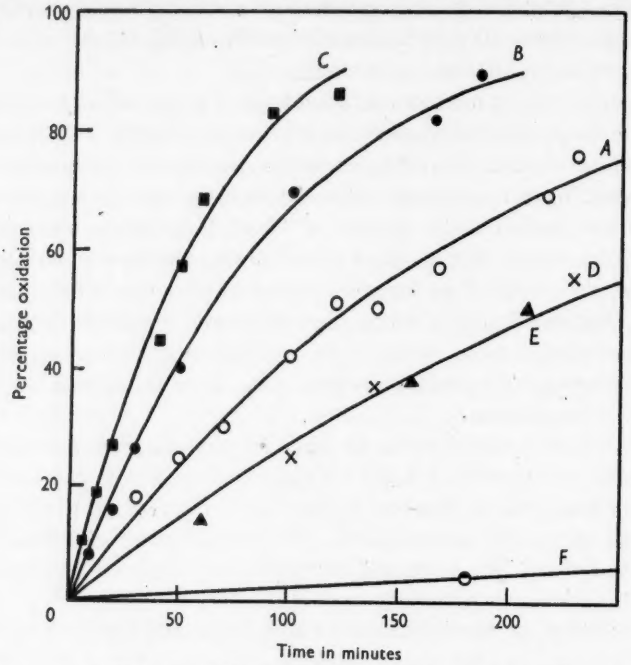


Fig. 1. *A*, butter serum of 30 August 1948, temp. 48°. *B*, butter serum +0·30 p.p.m. Cu, temp. 48°. *C*, butter serum +8·32 p.p.m. Fe, temp. 48°. *D*, butter serum +10% NaCl, temp. 48°. *E*, buttermilk of 30 August 1948, temp. 48°. *F*, butter serum of 30 August 1948, temp. 0°.

Table 1. *Effect of added copper*

Temperature (° C.)	Copper added to serum	12. vii. 48 k_1	12. vii. 48 k_1 for 0·1 p.p.m. added copper	23. viii. 48 k_1	23. viii. 48 k_1 for 0·1 p.p.m. added copper	30. viii. 48 k_1	30. viii. 48 k_1 for 0·1 p.p.m. added copper
48	Nil	—	—	0·0066	—	0·0056	—
	0·30	—	—	0·0124	0·0019	0·0113	0·0019
	0·60	—	—	0·0164	0·0016	0·0190	0·0022
0	Nil	0·00037	—	0·00030	—	0·00026	—
	0·26	0·00103	0·00025	—	—	—	—
	0·30	—	—	0·00118	0·00029	0·00111	0·00028
	0·60	—	—	0·00202	0·00029	0·00200	0·00029

It can be seen that the increase in k_1 is proportional to the concentration of added copper within the experimental error. The average value of k_1 for 0·1 p.p.m. copper is 0·0019 at 48° C. and 0·00028 at 0° C. This gives a value of 6·78 for the ratio k_1 48°/k_1 0°, corresponding to an activation energy E, of 6900 calories.

The effect of added iron. Table 2 gives a similar set of results for added ferric iron. The increase in rate is again proportional to the concentration of added iron. The average value of k_1 for 1·0 p.p.m. iron is 0·0015 at 48°, and 0·000029 at 0°. This gives a k_1 48°/k_1 0° ratio of 51·75, corresponding to an energy of activation, $E = 14{,}300$ calories.

Table 2. *Effect of added iron*

Temperature (° C.)	Iron added to serum	16. viii. 48 k_1	16. viii. 48 k_1 for 1·0 p.p.m. added iron	23. viii. 48 k_1	23. viii. 48 k_1 for 1·0 p.p.m. added iron	30. viii. 48 k_1	30. viii. 48 k_1 for 1·0 p.p.m. added iron
48	Nil	0·0152	—	0·0066	—	0·0056	—
	4·16	0·0211	0·0014	0·0117	0·0012	0·0122	0·0016
	8·32	0·0285	0·0016	0·0208	0·0017	0·0180	0·0015
0	Nil	—	—	0·00030	—	0·00026	—
	4·16	—	—	0·00042	0·000029	0·00039	0·000031
	8·32	—	—	0·00056	0·000031	0·00048	0·000026

Effect of the copper and iron originally present. In Table 3 are shown k_1 values, together with figures for the copper and iron content, for a number of serums and buttermilk to which no metals had been added, either intentionally or otherwise. The copper and iron figures were obtained by analysis, and have been adjusted to allow for the dilution of 5 ml. of serum or buttermilk to 6 ml. in the reaction mixture. A consideration of the results

Table 3. *Metals originally present*

Date	Sample	Copper (p.p.m.)	Iron (p.p.m.)	k_1 48°	k_1 0°
12. vii. 48	Serum	0·28	2·61	0·0066	0·00037
12. vii. 48	Buttermilk	0·11	1·83	0·0026	0·00012
23. viii. 48	Serum	0·28	2·91	0·0066	0·00030
30. viii. 48	Serum	0·24	1·68	0·0056	0·00026
30. viii. 48	Buttermilk	0·13	1·20	0·0032	0·00013

shows that the rate is directly proportional to the copper concentration, and that the iron present can have little or no catalytic action. The catalytic activity of the copper is, however, somewhat different from that of added copper. The k_1 value for 0·1 p.p.m. copper is 0·0024 at 48° and 0·00011 at 0°. The value of the ratio k_1 48°/k_1 0° is 21·8, corresponding to $E = 11{,}200$ calories.

Comparison of the rate in serum and buttermilk. From the results in Table 3 it can be seen that the greater rate in the serum, compared with buttermilk, is due entirely to the concentration of the copper which occurs in the serum during churning. A similar concentration of added iron would likewise cause an increase in rate in the serum. In Table 4 are the results for serum and buttermilk, obtained from cream to which copper had been added

Table 4. *Copper added before churning*

Sample	Copper content (p.p.m.)	48° C. k_1	48° C. k_1 for 0·1 p.p.m. copper	48° C. Orig. copper calc. (p.p.m.)	0° C. k_1	0° C. k_1 for 0·1 p.p.m. copper	0° C. Orig. copper calc. (p.p.m.)
Serum	0·73	0·0152	0·00208	0·27	0·0016	0·000219	0·25
Buttermilk	0·44	0·0091	0·00207	0·15	0·00098	0·000223	0·15

prior to churning. The close correspondence of the k_1 values for 0·1 p.p.m. copper in the serum and in the buttermilk indicates that, on churning, the added copper is distributed in a very similar way to the original copper. As a check on the k_1 values previously obtained for 0·1 p.p.m. added and original copper, they can be used, together with the data above,

to calculate the original copper content of the serum and buttermilk. The values obtained agree closely with the analytical figures for other serums shown in Table 3. The distribution of copper and iron between the buttermilk and serum is further discussed below.

Effect of sodium chloride. Analar NaCl was added to the reaction mixture to give a final concentration of 3·3 and 10%, the latter approximating that found in the serum of normal salted butter. The copper concentration was 0·73 p.p.m.

The following results were obtained (temp. 48°):

serum $k_1 = 0{\cdot}0152$,

serum + 3·3% NaCl $k_1 = 0{\cdot}0119$,

serum + 10% NaCl $k_1 = 0{\cdot}0080$.

In another experiment 10% NaCl was added to:

A. Serum containing no added metal (copper content 0·24 p.p.m.),

B. Serum + 0·3 p.p.m. copper,

C. Serum + 4·16 p.p.m. iron.

The resulting k_1 values (temp. 48°) are given in Table 5. From these results it appears that the reaction catalysed by the original copper and that catalysed by the added iron are reduced to about three-fifths of their former value, while the catalytic activity of added copper is reduced to about one-eighth.

Table 5. *Effect of sodium chloride*

Sample	k_1	k_1 for added copper	k_1 for added iron
A	0·0056	—	—
B	0·0113	0·0057	—
C	0·0122	—	0·0066
A + 10% NaCl	0·0033	—	—
B + 10% NaCl	0·0040	0·0007	—
C + 10% NaCl	0·0071	—	0·0038

These results must be regarded as preliminary only. Mapson (23) has studied in some detail the effect of NaCl on the reaction catalysed by ionic copper, and has found the rate to depend in a rather complicated way on the copper concentration, chloride ion concentration, pH, and buffer system. It is probable that a similarly complicated effect occurs in butter serum. Insufficient work has been done to warrant further discussion of the reactions involved.

Experiments at 20° C. In addition to the results reported above for 0° and 48°, a number of experiments were done at 20°, but anomalous results were obtained. It was found that the k_1 values decreased rapidly as the reaction proceeded. Further, in some cases, including all those in which ferric iron had been added to the reaction mixture, the initial titrations were greater than expected from the amount of ascorbic acid added, and the titration values further increased with time. It is probable that bacterial action was responsible for these effects. Bacteria could slow down the reaction by reducing the pH and the oxygen concentration. The high initial titrations and the increase with time were apparently due to the reduction of ferric iron to ferrous iron, which is titratable by the dichloro-phenol dye in meta-phosphoric acid.

The stability of ascorbic acid in milk, which had been inoculated with various strains of lactic acid bacteria, was recently studied by Roine (24). He found that in the presence of

sufficient bacteria the ascorbic acid was almost completely protected from oxidation. The oxidation-reduction potential was also measured, and Roine concluded that in milk the stability of ascorbic acid was dependent mainly on the oxidation-reduction potential, and that the pH had little effect. His results, however, are equally consistent with the view that the inhibitory effect of the bacteria is due to their ability to use up oxygen dissolved in the milk, and that the lowering of the oxidation-reduction potential is merely a parallel consequence of this. His failure to observe differences in the stability of the ascorbic acid at different acidities was also due probably to the lack of oxygen in the milk. Roine further observed that some reduction of dehydro-ascorbic acid to ascorbic acid occurred when the milk was inoculated with starter bacteria (compare Stewart & Sharp's experiments with *E. coli*). This effect may have taken place to a small extent in the experiments at 20° reported in the preceding paragraph.

DISCUSSION

The results show that in serum and buttermilk, uncontaminated with copper or iron, the rate of oxidation of ascorbic acid is directly proportional to the copper naturally present, and no evidence has been obtained of the operation of any other factor. However, as the cream used was from a herd of ten to twenty cows, and the experiments were confined to July and August, the results do not entirely eliminate the possibility that the stability of ascorbic acid may vary in milk from individual cows, or in milk produced at different seasons of the year, through being affected by factors other than the copper content.

In serum and buttermilk, contaminated with copper and iron, it has been shown that the oxidation of ascorbic acid is the result of three distinct concomitant reactions: one catalysed by the *original* copper, and having, for 0·1 p.p.m. copper, a k_1 value at 0° of 0·00011 and activation energy of 11,200 calories; one catalysed by *added* copper for which k_1 for 0·1 p.p.m. at 0° is 0·00028 and the activation energy is 6900 calories; and one catalysed by *added* ferric iron having for 1·0 p.p.m. iron a k_1 value at 0° of 0·000029, and activation energy of 14,300 calories.

It follows that at the temperature at which butter is normally stored (−13°), added copper would play the predominant part in the catalysis. In the amounts normally found iron would be of no importance, as at this temperature 0·1 p.p.m. copper would be approximately twenty times as active as 1·0 p.p.m. iron. With a rise in temperature, the iron-catalysed reaction, with the higher activation energy, is relatively favoured and is responsible for an increased, though still small, contribution to the total reaction. Thus at 20° 1·0 p.p.m. iron would be about one-fourth as effective as 0·1 p.p.m. copper.

It is of interest to estimate approximately the rate of disappearance of ascorbic acid from butter under these different conditions. Consider a fair average creamery butter, containing 0·15 p.p.m. copper and 1·00 p.p.m. iron. The concentration of metals in the serum would be approximately 0·83 p.p.m. copper (say 0·33 p.p.m. original and 0·50 p.p.m. added) and 5·55 p.p.m. iron (say 3·00 original and 2·55 added). By using the Arrhenius equation it can be calculated that at −13°, k_1 would equal 0·00094. To this value, added copper would contribute 0·0008, original copper 0·00012 and added iron 0·00002. The time taken for half the ascorbic acid to be oxidized would be about 12 hr. At 20° the value of k_1 (added copper 0·0035, original copper 0·0015, added iron 0·0005), and the corresponding time for half oxidation is about 2 hr. These results do not take into account the inhibitory

effect of bacterial action or of sodium chloride. In salted butter the above times might be increased to twice the values shown.

In view of the kinetic results obtained, it is appropriate to discuss what is known of the chemical state in which copper and iron exist in milk and related products. This has been the subject of a number of investigations, the results of which are considered in the following four paragraphs.

(i) Davies (14) measured, potentiometrically, the concentration of ionic copper in milk, to which a copper salt had been added. His results showed that the fraction of total copper ionized increased with increase in acidity, but was extremely low at all acidities met with in dairy products.

(ii) The same author (*loc. cit.*), by comparing the copper content of the cream and skim milk, obtained from whole milk by gravity separation and by centrifuging, showed conclusively that the metal tends to distribute itself according to the effective absorbing area of the fat globules.

(iii) The nature of the fat-globule membrane or envelope has been studied extensively by Palmer and his collaborators. Palmer & Wiese (15) have found the material adsorbed at the fat-globule surface to contain a protein, not identifiable with other milk proteins, and a mixture of phospholipids. That the fat-globule envelope is a lipo-protein complex is confirmed by Moyer (16) from electrophoretic experiments. Jenness & Palmer (17), in experiments with washed cream, have shown that, on churning, the envelope material is concentrated in the butter serum, as compared with the buttermilk. It is therefore to be expected that the copper and iron would be similarly concentrated. Davies (*loc. cit.*), using cream to which copper had been added (25 p.p.m.), found that this was so, and that the concentration of copper in the serum was approximately twice that in the buttermilk.

The experiments done in connexion with the present work gave ratios of serum/buttermilk for copper of 2·2, 1·6, 1·8 and 2·5, while the corresponding figures for iron were 2·1, 1·5, 1·4 and 1·4. In normal factory practice larger and more variable ratios have been found, chiefly due to different churning conditions, including the addition of water at various stages and also due to sampling errors and irregular distribution of metallic particles. The buttermilk was sampled half-way through the run-off, while the butter samples were obtained from one box from each churning. Further metallic contamination of the butter after the buttermilk was run off would also increase the ratio. This is shown in Tables 6 and 7, where the results are given of analyses done in this laboratory on creamery butters and the corresponding buttermilks from different factories at various times.

Table 6. *Distribution of copper (p.p.m.)*

Sample no.	Buttermilk copper	Butter		Ratio: $\frac{\text{Serum}}{\text{Buttermilk}}$
		Copper	Copper in serum	
1	0·30	0·27	1·46	4·9
2	0·30	0·19	1·03	3·4
4	0·14	0·13	0·70	5·0
5	0·31	0·22	1·19	3·8
6	0·12	0·11	0·59	4·9
7	0·21	0·14	0·76	3·6
8	0·31	0·19	1·03	3·3
9	0·21	0·15	0·81	3·9

In the figures for butter, the first column gives the analytical results expressed as parts of copper or iron per million parts of sample. The second column of figures represents

parts of metal per million parts of serum, and have been calculated from those in the first column, on the assumption that the butters contained 18·5% serum. This concentration of copper and iron in the butter serum during churning may account for the fact that the serum has a higher oxidation-reduction potential than the corresponding buttermilk as found by Dutch workers (6).

Table 7. *Distribution of iron (p.p.m.)*

Sample no.	Buttermilk iron	Butter		Ratio: Serum / Buttermilk
		Iron	Iron in serum	
3	1·14	1·37	7·40	6·5
4	0·57	0·52	2·87	5·0
5	1·28	1·17	6·32	4·9
6	1·24	0·60	3·24	2·6
7	1·50	0·68	3·67	2·4
8	1·16	0·90	4·86	4·2
9	1·32	1·02	5·51	4·2

(iv) McIlroy (18) has examined the distribution of copper in milk by analysing fat and protein fractions. He found 'that the copper was combined almost entirely with the protein (soluble and insoluble), traces only being found in the fat. No free copper was detected in the serum.' Van der Waarden (19) separated butter into four fractions—fat fraction, protein fraction, phospholipid fraction and water fraction. Each fraction was then analysed for copper and iron. The fat fraction contained no metals, the protein fraction contained all the iron and 50–75% of the copper, while the phospholipid fraction contained 25–50% of the copper. No iron and only a small amount of copper was found in the water fraction. Added copper, if less than 100 μg., and added iron, if less than 750 μg., per serum fraction from 50 g. of butter, was distributed in the same way as the original. The butter was unsalted, and made from acid cream.

All this evidence establishes beyond doubt that copper and iron do not exist in the ionic state in milk and related products, but are combined in some way with the lipo-protein complex which surrounds the fat globules. From this it follows that the catalytic action of these metals must be considered as the effect of the combined metal, and not as the effect of the ion. The type of compound involved is probably similar to that studied by Stotz, Harrer & King (20), who added copper sulphate to different proteins and obtained complexes which catalysed the oxidation of ascorbic acid. Various organic complexes of ferric iron have also been found to act as catalysts in this oxidation (21).

The kinetic results, obtained in the present work, show that a distinction must be made between the states in which the added and original metals exist. This may mean either that the original and added metals are combined with different compounds or that they are attached to one compound in two different ways. In view of the results shown in Table 4, which indicate that the original and added copper are distributed in the same way on churning, the latter alternative seems more probable.

Dills & Nelson (22) have reported the isolation of a copper protein (copper 0·19%; nitrogen 15%) from skim milk, which they state has no ascorbic acid oxidase activity. If the catalytic powers of the copper remained unchanged during the isolation of the protein, correlation of their findings with the kinetic results reported here is difficult. Perhaps the catalytic power of the copper-protein complex is dependent to some extent on its colloidal state, so that it is lost more readily during isolation than that of a true enzyme.

Because of the difference in the results obtained by different investigators, little is to be gained by trying to compare in detail the kinetics of the oxidation of ascorbic acid catalysed by ionic copper, with the results obtained here for the catalysis by copper protein complexes in butter serum. Generally speaking, the rate of the reaction in butter serum is slower than that due to ionic copper.

SUMMARY

1. The determination of ascorbic acid in butter by the usual macrotitration method was found to be impossible because this substance rapidly became oxidized while the butter was being melted, so that only about half could be recovered.

2. A simple micro-titration procedure enabled more reliable results to be obtained, but the loss of ascorbic acid from smaller samples could not be reduced below about 10% under the most favourable conditions.

3. Kinetic experiments have been carried out on butter serum and buttermilk to which ascorbic acid had been added, and from the results it has been deduced that the oxidation of ascorbic acid takes place by three distinct concomitant reactions: one catalysed by the original copper, one catalysed by added copper, and one catalysed by added ferric iron.

4. It has been calculated that in an unsalted butter of normal metallic content half the ascorbic acid would be oxidized in about 12 hr. at cold storage temperature (−13° C.). At this temperature, 0·1 p.p.m. of added copper is about twenty times as effective a catalyst as 1·0 p.p.m. of added iron. At room temperature the corresponding half-oxidation time would be about 2 hr., and here 0·1 p.p.m. of added copper is about four times as active as 1·0 p.p.m. of added iron. In salted butter the inhibitory effect of the chloride ion would increase these times somewhat.

5. The chemical state in which copper and iron exist in milk and related products has been discussed, especially in relation to the kinetic results obtained.

The author is indebted to Dr G. M. Moir for helpful advice and criticism throughout the course of the work and in the preparation of the manuscript; to Mr G. I. Thomson for assistance with the churning and buttermaking; and to Mr F. Bishop who analysed the butter serum and buttermilk for copper and iron.

REFERENCES

(1) Brown, W. C. & Thurston, L. M. (1940). *J. Dairy Sci.* **23**, 629.
(2) Beck, G. H., Whitnah, C. H. & Martin, W. H. (1939). *J. Dairy Sci.* **22**, 17.
(3) Leeder, J. G. & Herreid, E. O. (1942). *Bull. Vt agric. Exp. Sta.* no. 481.
(4) Olson, F. C. & Brown, W. C. (1942). *J. Dairy Sci.* **25**, 1027.
(5) Krukovsky, V. N. & Guthrie, E. S. (1945). *J. Dairy Sci.* **28**, 565.
(6) *Saal, R. N. J. & Heukelom, W. (1946). *Versl. RijkslandbProefst., 's Grav.*, no. 52G (3), 99.
(7) Stewart, A. P. & Sharp, P. F. (1945). *Industr. Engng Chem.* (Anal. ed.), **17**, 373.
(8) Sharp, P. F., Hand, D. B. & Guthrie, E. S. (1941). *Industr. Engng Chem.* (Anal. ed.), **13**, 593.
(9) Perrin, Dawn R. & Perrin, D. D. (1946). *N.Z. J. Sci. Tech.* **28**, 266A.
(10) Mawson, E. H. & Kon, S. K. (1945). *Lancet*, **259**, 14.
(11) Doan, E. J. & Josephson, D. V. (1943). *J. Dairy Sci.* **26**, 1031.
(12) Farmer, G. I. & Abt, A. F. (1936). *Proc. Soc. exp. Biol., N.Y.*, **34**, 146.

* See also *Research in Holland: Chemical and Physical Investigations on Dairy Products* (1947), by Eilers, Saal and van der Waarden (Elsevier Publishing Co., Amsterdam).

(13) DEATH, G. C., LIGHTFOOT, F. RUTH & MOIR, G. M. (1947). *N.Z. J. Sci. Tech.* **28**, 273A.
(14) DAVIES, W. L. (1933). *J. Dairy Res.* **4**, 255.
(15) PALMER, L. S. & WIESE, H. F. (1933). *J. Dairy Sci.* **16**, 41.
(16) MOYER, L. S. (1940). *J. biol. Chem.* **133**, 29.
(17) JENNESS, R. & PALMER, L. S. (1945). *J. Dairy Sci.* **28**, 611.
(18) MCILROY, R. J. (1935). *N.Z. J. Sci. Tech.* **17**, 710.
(19) *VAN DER WAARDEN, M. (1944). *Versl. RijkslandbProefst., 's Grav.*, no. 50 G (2), 61.
(20) STOTZ, E., HARRER, C. J. & KING, C. G. (1937). *J. biol. Chem.* **119**, 511.
(21) NIKOLAEV, L. A. (1947). *Věstn. Moskov. Univ.* no. 7, 147. (Cited from *Chem. Abstr.* (1948), **43**, 3654.)
(22) DILLS, W. L. & NELSON, J. M. (1942). *J. Amer. chem. Soc.* **64**, 1616.
(23) MAPSON, L. W. (1941). *Biochem. J.* **36**, 494.
(24) ROINE, P. (1948). *Acta Chem. Scand.* **2**, 97.

* See note, p. 64.

(*MS. received for publication* 3 *October* 1949)

637.22

411. *PSEUDOMONAS PUTREFACIENS* IN NEW ZEALAND BUTTER

By G. I. THOMSON
Dairy Division's Laboratory, Wallaceville, New Zealand Department of Agriculture

INTRODUCTION

A number of investigations on *Pseudomonas putrefaciens* and its relation to the 'surface taint' defect in butter have been reported over a period of years from the United States, where the organism was first isolated from defective butter in 1931 by Derby & Hammer (1). In Canada, where the 'surface taint' defect was first noted in 1919, comprehensive reports on *Ps. putrefaciens* have recently been published by Wolochow, Thornton & Hood (2). In western Australia, Cullity & Griffin (3) recorded the occurrence of 'surface taint' defect due apparently to *Ps. putrefaciens*, and similar work has been reported by Itzerott (4) and by Pont (5), who reviewed earlier investigations by Loftus-Hills and others in eastern Australia, where the defect has been commonly spoken of as 'rabbito'.

In New Zealand there have been reports from time to time of surface-flavour defects, but these have rarely, if ever, been studied in sufficient detail to enable any particular organism to be identified as the prime cause. The only case recorded is contained in a report of Gilruth (6), who isolated an organism, apparently *Bacillus fluorescens liquifaciens* (*Pseudomonas fluorescens*), from the water supply of a factory having trouble with flavour defects. When inoculated into butter and into laboratory media and incubated at 45° F., this organism produced a 'peculiar foetid' odour; it also remained viable in butter stored at below freezing-point for 6 months. There are no records of attempts to isolate *Ps. putrefaciens* from either defective or normal butter. In order then to determine the presence or otherwise of this organism in New Zealand butter, a preliminary survey was undertaken of samples from some ninety-four brands received from seven export grading ports during the past season, and the results are recorded in this paper.

METHODS

Hammer's enrichment technique

The methods used in this investigation are based on the enrichment technique developed by Long & Hammer (7). Enrichment is necessary because of the difficulty experienced in isolating *Ps. putrefaciens* on ordinary media, due to its poor growth and the ease with which it is overgrown by other bacteria. Hammer's technique consists of inoculating the butter or butter serum into tubes of litmus milk, incubating them at 3° C. until reduction of the litmus occurs. The cultures are then streaked on a special iron gelatine agar medium which has the following composition:

	%
Gelatine	4·0
Proteose peptone	2·0
Dipotassium phosphate	0·1
Ferric ammonium citrate	0·05
Agar	1·5
Water, to make	100·0

On this medium *Ps. putrefaciens* grows exceptionally well, and most strains develop sufficient colour in 48 hr. to enable a reasonably accurate identification of the colonies by visual examination. Owing to a shortage of proteose peptone it was found necessary to substitute Difco peptone, without any appreciable effect on the results.

Adaptation of the technique

For many years the bacteriological examination of butter in this laboratory has been done by a modified 'Frost little-plate' method (8). Before using the enrichment technique an attempt was made to isolate *Ps. putrefaciens* by 'fishing' likely colonies from these 'little-plate' cultures. Only two out of forty-two colonies proved on further identification tests to be typical *Ps. putrefaciens*, so this method was abandoned.

Hammer's method was then adapted to the routine testing by adding chilled sterile litmus milk to what remained of the 1 in 10 dilution of butter (i.e. 1 g. of butter in 9 ml. of saline) after completion of the culturing operations. The test-tubes, containing about 7·5 ml. of butter dilution plus about 8 ml. of sterile litmus milk, were then incubated in a refrigerator at 6° C. until reduction of the litmus milk took place. The decolorized cultures were then streaked on dry plates of Hammer's medium and incubated at 22° C. for 48 hr. Colonies of *Ps. putrefaciens* were very readily identified on this medium, but in order to make certain typical colonies were 'fished' under a binocular microscope and sub-cultured into litmus milk. Rapid reduction of the litmus in about 12 hr. at 22° C. confirmed the identification.

Little success was obtained at first, until several minor details were given due attention. First, both the litmus milk and the butter dilution must be chilled to at least 6° C. before mixing and placing in the refrigerator. Second, the organism seems to grow readily only in the very surface fatty layer of the litmus milk, so the loopful for streaking must be taken from this part of the culture without the usual preliminary shake. Many other species of bacteria, particularly of the Pseudomonas group, were found to grow in litmus milk at 6° C. and reduce it, but decolorization was inclined to be patchy with frequently a ring of blue left at the surface. Cultures later found to contain *Ps. putrefaciens* were invariably completely decolorized, with a quite yellow layer of butterfat at the surface.

The enrichment time and temperature

Over 90% of positive isolations were made from those litmus milk enrichment cultures which were reduced during 8 days' incubation or less at 6° C. The times taken for reduction in ninety-one cases were as follows: 5 days or less, 32 cultures; 6 days, 18 cultures; 7 days, 25 cultures; 8 days, 8 cultures; over 8 days, 8 cultures.

There appeared to be no advantage in prolonging the incubation at this temperature, as the majority of the cultures were wholly or partially decolorized within a fortnight. Also, the occasional positive isolation obtained after 8 days' incubation did not warrant the time and media needed. Hammer recommended an incubation temperature of 3° C. and a period of up to a fortnight. This combination of longer time with lower temperature would perhaps yield a higher percentage of positive isolations.

RESULTS

During a period of 6 months, a total of 1563 samples of butter were examined using the technique described. It should be emphasized that every sample examined was taken

from normal salted butter as graded for export, with salt usually in the range 1·5–2·0%. A total of 321 litmus milk enrichment cultures were decolorized after incubation at 6° C. and were plated out on the iron gelatine agar, and from these ninety-one positive cultures were obtained.

Pseudomonas putrefaciens *and factory hygiene*

A feature of the butter samples from which *Ps. putrefaciens* cultures have been isolated is that there is obviously a close correlation between poor factory hygiene and the incidence of this organism in the butter. Out of ninety-one samples from which isolations were made as described, seventy-seven were classed on bacteriological counts as 'poor' or 'bad'. Specifically, sixty-nine had total bacterial counts over 50,000 per g., fifty-three had yeast counts classed as 'bad' (over 500 per g.) and forty-six had coliform contamination either 'poor' or 'bad'.

The incidence of contamination of butter with *Ps. putrefaciens* on a district basis naturally lines up with the above. In other words, those districts with a generally higher standard of factory hygiene (as demonstrated by bacterial counts) have a relatively low incidence, whereas districts with lower hygienic standards have a much higher incidence. As regards individual factories, in some cases *Ps. putrefaciens* has been isolated almost every time the butter has been examined, while other factories have given positive results only spasmodically. There has not so far been any opportunity to track down the major source of contamination in the factories affected. In one case the water supply was found to contain the organism. However, most of the results obtained suggest that the major source is unclean equipment—as is strongly suggested by the following case. Of twelve churnings of cG9 butter made between 5 January and 14 February, eight had counts of over 100,000 organisms per g., and of these five were positive for *Ps. putrefaciens*. The last positive result was from the first churning of 14 February, the total count being over 100,000 organisms per g. The first churnings of 15 and 16 February were also examined and the total counts were 18,700 and 23,300 organisms per g. respectively. Of the following seventeen churnings tested, none had counts over 100,000 and only four were over 50,000 per g. None of these seventeen churnings was positive for *Ps. putrefaciens*. A pocket of contamination was apparently got rid of on 14 February, and with it the main source of the *Ps. putrefaciens* contamination. This case demonstrates the important part the laboratory can play in improving factory hygiene and thereby preventing defects such as surface taint. As Itzerott has pointed out, 'An outbreak of "rabbito" is invariably preceded by a sudden rise in butter counts, hence the routine study of butter counts at regular intervals by laboratory technicians provides a most valuable and useful aid in predicting an outbreak' (4).

Biochemical reactions and variations

One or more cultures were obtained from thirty separate brands, and one culture from each brand was kept for further study. The following morphological and biochemical observations were made upon each culture.

(1) Growth and colour production on Hammer's medium.
(2) Reduction of litmus milk, followed by a brown coloration and proteolysis.
(3) Phosphatase production in litmus milk.
(4) Gram staining, motility, etc.
(5) Effect on butter (only a few of the cultures were inoculated into butter).

Proteolysis of litmus milk cultures by *Ps. putrefaciens* liberates phenolic substances or other groupings which react to give the blue indophenol colour with Folin and Ciocalteau's reagent as used in Neave's modification of Kay and Graham's phosphatase test (10). It is therefore necessary, when demonstrating phosphatase production, to carry out a control test on each litmus milk culture to be tested. This control test, which measures any colour produced by interfering substances, is done by completing the test without incubating the buffer substrate and the litmus milk culture. The Folin and Ciocalteau's reagent is added to the buffer substrate solution, followed then by the litmus milk culture, and the test completed in the normal way.

The colour produced in these control tests on litmus milk cultures of *Ps. putrefaciens* increases appreciably while the cultures are growing, and it is therefore desirable to carry out the phosphatase test within 24–48 hr. from the time the litmus milk is inoculated with the culture under examination.

Considerable variations were observed in colour production, litmus reduction, proteolysis, rate of growth, etc., but none that has not been fully described by Hammer (7). Only one culture showed marked differences as follows:

(1) Very slow growth and less than normal colour production on iron gelatine agar medium.

(2) Very slow reduction of litmus milk—taking weeks instead of hours.

(3) In litmus milk little formation of brown colour and little proteolysis which normally occurs after reduction.

(4) No phosphatase formation.

(5) Numerous involution forms present even in a young culture.

(6) A slow but definite formation of the putrid-cheesy defect in butter.

This culture was isolated from a 'Frost little-plate' culture of wN butter on one occasion only; the fact that it was a gram-negative bacillus which could produce the typical defect in butter suggested that it might be a variant of *Ps. putrefaciens*.

A similar organism was isolated in the same way (from a 'Frost little-plate' culture) from a sample of vM butter. When first isolated the organism grew slowly on the gelatine agar and formed less colour then normal cultures and, although it reduced litmus milk very slowly, its rate of proteolysis in this medium was above average. Phosphatase formation was normal but the production of the defect in butter was also slow. Later on in the season the organism was repeatedly isolated from vM butters in the routine enrichment tests. Distinct changes in colony appearance and biochemical characteristics of the organism were observed to be taking place, and these differences were still exhibited by subcultures after a period of several months. Although, at first, one of the slowest growing and weakest colour producers, later isolations of the vM strain yielded one of the fastest growing and most highly coloured in the collection. The organism was usually isolated from the first and third of the daily churnings, both of which invariably had high bacterial and yeast counts in contrast to the second churning done in a different churn. As an examination of the factory water supplies and chilled water failed to reveal the presence of the organism it was probably derived from some pocket of contamination associated with no. 1 churn or its equipment. To explain the variations noted above, the suggestion is put forward that they were due to the effect of environment. Finding itself with an abundant source of suitable food (i.e. cream as compared with meagre food supplies of its natural habitat, soil or water), and conditions entirely favourable to maximum growth

(i.e. moisture, optimum temperatures, etc.) the organism flourished so prolifically that it developed its morphological and biochemical characteristics to the fullest extent.

DISCUSSION

Although the defect known as 'surface taint' is rarely identified in the butter at the time of grading (3–7 days after manufacture), *Ps. putrefaciens* is evidently far more common in New Zealand butter than has been realized. Butter, from which *Ps. putrefaciens* has been isolated repeatedly and quite readily, has graded well, so it seems evident that as Hammer has pointed out, the mere presence of the organism in the butter does not necessarily lead to a development of the defect because various factors restrain its growth, namely salting, thorough working and low-temperature storage. Writing of Victorian export butter, Itzerott says that 'Butters contaminated with "rabbito" organisms have frequently escaped the notice of graders (probably due to the fact that the butter has not been subjected to conditions favourable to the development of any stages of the defect prior to grading) only to develop the defect on arrival in England after removal from cold storage and thawing out' (4).

The following case shows that high quality salted butter can, under favourable conditions, develop 'surface taint'. The manager of one of the highest grading factories noticed that his butter developed an objectionable odour when kept at room temperature for several days, but no defect developed during cool storage. A sample of butter submitted for examination failed to develop the defect and no surface taint organisms were isolated by the enrichment technique. But an examination of the factory's water supply showed the presence of *Ps. putrefaciens* in the well supplying the chilled-water tank, and later on in the season the organism was isolated from the butter itself.

It is quite likely therefore that in the retail sale of butter, both in New Zealand and in England, there are occasions when butter containing *Ps. putrefaciens* is exposed at suitably warm temperatures long enough to allow the development of 'surface taint' due to this organism. This might explain some of the defects of retail butter complained of by the New Zealand housewife, particularly in past years before the fairly general use of refrigerators in retail stores. To ascertain the incidence and effect of this and other undesirable bacteria upon butter as used by the consumer, the best method would be to arrange storage trials in which butter was kept at normal air temperature (60–70° F.) for, say, 4 weeks prior to examination in the usual way for flavour, etc.

Further investigations are desirable to locate the source of the contamination in the factories making butter from which *Ps. putrefaciens* has been isolated. Attempts to isolate this organism from infected butter after a period of cold storage should provide information about the viability of the organism under these conditions. Even if the organism cannot be isolated after a period of storage at low temperature it may already have adversely affected the flavour. In a recent report Elliker & Horrall (9) describe experiments to show the effect of *Ps. putrefaciens* upon diacetyl and the flavour of butter. They state that 'the first apparent stage of decomposition by this organism was a complete loss of typical butter aroma. The butter became flat in odour and flavour'; also 'Results and observations indicate that the ability of *Ps. putrefaciens* and other organisms to produce a flat-flavoured butter is perhaps more prevalent than is commonly realized'.

SUMMARY

1. Using Hammer's litmus milk enrichment technique, a total of ninety-one cultures of *Ps. putrefaciens* were isolated from 1563 samples of normal salted butter graded for export.

2. A correlation was demonstrated between poor hygienic conditions in butter factories and the incidence of *Ps. putrefaciens* contamination of the butter.

The author wishes to express his appreciation to Dr G. M. Moir for his interest in the work and his assistance in preparing the manuscript.

REFERENCES

(1) DERBY, H. A. & HAMMER, B. W. (1931). *Res. Bull. Ia agric. Exp. Sta.* no. 145.
(2) WOLOCHOW, H., THORNTON, H. R. & HOOD, E. G. (1942). *Sci. Agric.* **22**, nos. 5–10.
(3) CULLITY, M. & GRIFFIN, D. G. (1938). *J. Dep. Agric. W. Aust.* **15**, 137.
(4) ITZEROTT, G. (1941). *J. Dep. Agric. Vict.* **39**, 39.
(5) PONT, E. G. (1941). *J. Coun. sci. industr. Res. Aust.* **14**, 1.
(6) GILRUTH, J. A. (1899). *N.Z. Dep. Agric. 7th Ann. Rep.* p. 89.
(7) LONG, H. F. & HAMMER, B. W. (1941). *Res. Bull. Ia agric. Exp. Sta.* no. 285.
(8) MOIR, G. M. & RUSSELL, R. R. (1939). *J. Dairy Res.* **10**, 310.
(9) ELLIKER, P. R. & HORRALL, B. E. (1943). *J. Dairy Sci.* **26**, 943.
(10) NEAVE, F. K. (1939). *J. Dairy Res.* **10**, 475.

(*MS. received for publication* 17 *October* 1949)

637.22

412. COLIFORM BACTERIA IN NEW ZEALAND BUTTER

By G. I. THOMSON

Dairy Division's Laboratory, Wallaceville, New Zealand Department of Agriculture

INTRODUCTION

The detection of coliform types of bacteria in butter is an important feature of the bacteriological control of butter factory hygiene, because of their harmful effects on flavour (1). These bacteria are also useful 'indicator organisms' of manufacturing efficiency, for they are completely destroyed at pasteurizing temperatures of much below 200° F., so their presence in butter means that at some stage in the manufacturing process, some operation has not received due care or attention. Providing that pasteurizing is effective, the presence of coliform bacteria is usually due to some part of the equipment not being properly cleaned. The coliform contamination depends on residual traces of milk solids and is thus essentially indirect.

The three cardinal requirements of any presumptive coliform test used on large numbers of samples are simplicity, accuracy and economy. The test most widely used in British countries employs a bile salt-lactose broth medium, usually called MacConkey broth, the merits of which have been fully discussed by Wilson (2). He found that this medium gave few false positive results from water or milk. But as Grimes (3) points out, 'it is known that this medium favours the growth of the *Escherichia*' (faecal type), whereas several workers, particularly Hammer & Yale (4), have found that more harm is done to butter flavour by the *Aerobacter* (or *aerogenes*) group of coliform bacteria.

When the routine testing of butter was instituted in this laboratory some years ago (5), the method adopted for the detection of coliform contamination was a modification of Grimes's method. Because of its simplicity and economy, and also because it did not discriminate against the *aerogenes* group, this appeared to have advantages over the standard presumptive test employing MacConkey broth.

The method consists of adding 1 ml. of a 1 in 10 dilution of butter to each of two tubes of sterile litmus milk and incubating one tube at 37° C. and the other at 20° C. (to detect those *aerogenes* types unable to form gas at 37° C.). The presence of coliform bacteria is indicated by the appearance of bubbles and cracks caused by gas which is held in the clotted milk. Grimes confirmed positive results in these presumptive tubes by streaking on eosin methylene-blue agar.

One modification adopted in this laboratory was that of inoculating one tube of litmus milk only and incubating at 30° C., as it was found that this still ensured the detection of those *aerogenes* types which do not form gas at 37° C. (Hiscox (6) and Oxley (7) both found that some strains of coliform organisms can be detected using MacConkey broth by incubation at 30° C. but not at 37° C.). Secondly, owing to the large numbers of samples being handled by the limited staff available, the confirmatory part of this method had to be omitted.

Compared with the MacConkey broth medium, the use of litmus milk has several advantages:

(1) It is cheap and easily prepared.

(2) It was found by Grimes to be a better enrichment medium than nutrient lactose broth for the detection of organisms of the *coli-aerogenes* group in butter.

(3) It provides a rough quantitative estimation of the numbers of coliform organisms present. Thus according to the numbers of cracks and gas bubbles showing in the clotted milk, the sample may be put into one of four grades—coliform germs 'absent', 'trace', 'present', or 'much present' as the case may be.

It was realized that the use of the litmus milk in this way without confirmation might result in a greater degree of inaccuracy as compared with the use of MacConkey broth. The purpose of this present investigation then was to determine the reliability of the simplified litmus milk test for coliform organisms in butter. All the samples examined were salted butter, with salt usually in the range 1·5–2·0%.

EXPERIMENTAL

In the first part of the investigation all litmus milk cultures classed as positive, i.e. coliform 'trace', 'present' or 'much present', were subcultured into MacConkey broth tubes. In addition, a smaller number of doubtful positive tubes (i.e. those which might be 'trace' but which were actually recorded as 'absent') were also subcultured in a like manner. In each case about 1 ml. of milk culture taken from an area showing gas bubbles was transferred to a tube of MacConkey broth, using a sterile Pasteur pipette for each sample. Those cultures showing acid and gas after incubation for 48 hr. at 30° C. were confirmed positive by streaking on brom thymol blue agar (8).

Table 1. *Confirmation of presumptive tests*

Classification	Interpretation	No. of samples	No. confirmed positive	No. of false positives
Much present	Bad	7	7	Nil
Present	Poor	94	81	13
Trace	Fair	83	35	48
Doubtful trace*	—	85	10	—
Absent	Good	403	—	—

* Recorded as absent.

The results on 672 samples are shown in Table 1. To interpret the coliform results when advising the factory staffs for instructional purposes, the terms shown in the second column of Tables 1 and 2 are used. None of the 403 samples classed as 'absent' was subcultured in MacConkey broth. As the number of false positives particularly in the 'trace' category seemed extraordinarily high, a further 401 samples were examined in the same way, but a stricter differentiation was made between 'trace' and 'absent'. The results are shown in Table 2.

Table 2. *Confirmation of presumptive tests*

Classification	Interpretation	No. of samples	No. confirmed positive	No. of false positives
Much present	Bad	4	3	1
Present	Poor	68	62	6
Trace	Fair	25	19	6
Doubtful trace*	—	74	56	—
Absent	Good	230	—	—

* Recorded as absent.

The figures from both Tables 1 and 2 include 1073 samples, and show that of 267 samples

classed as 'trace' or 'doubtful trace', only 120 were confirmed positive. Thus when very few bubbles or cracks are present (i.e. 'trace' category), the chances are only about 1 in 2 that organisms of the *coli-aerogenes* group are present. These results made clear the difficulty of 'reading' the tubes showing only traces of gas formation. Again three observers did not agree in the results they recorded. Another important factor is that Hassouna & Allen (9) have shown that gas-production in the *coli-aerogenes* group takes place only in the initial stages of growth. Yet in a butter with a low total count, clotting of the milk may be delayed for long enough to allow most of the gas formed to escape. On the other hand, a butter with a higher count may yield rapidly a good acid clot which has a marked tendency to contract and 'whey off' on prolonged incubation so that bubbles of gas may be lost. Double or triple reading of the tubes would be necessary to get over this effect.

In an endeavour to improve the accuracy of the presumptive part of Grimes's method, two modifications were tried in comparison with MacConkey broth on a further 719 samples of butter. The first modification was an adaptation of Orla-Jensen's (10) Rennet Fermentation test in which sufficient rennet to cause clotting was added to the litmus milk after inoculating the butter dilution. In the second modification a 1% concentration of agar was used to 'solidify' the milk. This 'litmus milk agar' medium is prepared as follows. Ten grams of agar are dissolved in 200 ml. of tap water and added to 700 ml. of hot fresh litmus milk. After mixing thoroughly, the medium is tubed while hot and sterilized by intermittent steaming. A similar method of demonstrating gas-production has been used by Huttig (11) to detect coliform contamination in milk used for cheese-making, and also by Minster (12), who used 'purple salt agar' and 'purple lactose agar' to detect gas-forming organisms in tests for the keeping quality of unsalted butter.

Comparative trials were carried out as follows: The 1 in 10 dilution (1 g. of butter in 9 ml. of saline) was warmed to 45° C. and, after thorough shaking, 1 ml. was inoculated into each of the following:

(1) One tube containing about 7 ml. of MacConkey broth and a Durham fermentation tube.

(2) One tube of litmus milk to which was added after inoculation sufficient strong rennet and sterile calcium chloride solution to ensure clotting in about 30 min.

(3) One tube of litmus milk agar which was melted and then held at 45° C. until inoculated, when the agar was immediately solidified by placing in cold water.

All the cultures were then incubated for 5 days at 30° C., but owing to the tendency of the renneted litmus milk to whey off and so lose the gas bubbles, these tubes were examined after 48 hr. and again at 5 days. The renneted litmus milk and the litmus milk agar cultures were classed as 'much present', 'present', 'trace', or 'absent', according to the number of bubbles and cracks, while the amount of gas in the MacConkey broth tubes was recorded as +++, ++, or +, the last being the minimum quantity of gas required to record as a positive result.

The presumptive positive results were confirmed by streaking a loopful from the MacConkey broth tube on a MacConkey agar plate. When only the renneted milk or milk agar cultures were positive, a small quantity of the milk culture was inoculated into MacConkey broth, incubated at 30° C. for several days and then, if positive, confirmed by streaking on a MacConkey agar plate. It was assumed that, when two or three of the three

cultures from the same sample were positive, the same organism was responsible for the gas production in each case.

RESULTS

Table 3. *Comparison of media*

Medium	Total samples	Confirmed positives		False positives	
		No.	%	No.	%
MacConkey broth	719	—	—	—	—
Over 10% of gas	—	137	19·05	Nil	—
Trace of gas	—	13	1·8	Nil	—
Renneted milk	434	—	—	—	—
Much present	—	7		3	
Present	—	79	30·2	10	3·0
Trace*	—	45			
Litmus milk agar	719	—	—	—	—
Much present	—	18		4	
Present	—	90	21·7	14	2·5
Trace	—	49			

* A further thirty-eight samples showing doubtful traces of gas were recorded as absent and were not subcultured.

Table 3 shows that the renneted milk produced the highest percentage of positive results, but also the highest percentage of false positives. The same difficulty in reading the 'trace' positives occurred as with the Grimes's medium. After 5 days' incubation there were frequently clusters of small bubbles present which were apparently not caused by coliform organisms and these made the reading of the tubes rather difficult. Incubation of control tubes of milk (with rennet only) showed that the rennet was to blame, but an attempt to sterilize it by filtration was not successful. Another difficulty, due to wheying off, also frequently occurred at the end of 5 days, but, although reading the tubes at 48 hr. obviated this difficulty, some tubes negative at 48 hr. appeared positive at 5 days. Another disadvantage of the renneted milk test was the necessity of adding the rennet and calcium chloride solution after the inoculation which meant double handling of the tubes.

The milk agar medium, while not yielding as many positive results as the renneted milk, gave fewer false positives. Although investigation of these in detail was not possible, it was noticed that, as might be expected, four-fifths of the false positives occurring with this medium were in tubes classed as 'trace'. For the purposes for which this routine testing is undertaken, this can be regarded as quite satisfactory. Further work is required to find out whether this medium gives significantly more positives than bile salt broth.

This litmus milk agar medium has been used in the laboratory for over a year, instead of litmus milk. As regards making the medium and culturing the butter samples, the time and labour needed have not been materially increased. The medium has several advantages over both litmus milk and renneted litmus milk, as follows:

(1) As there is no wheying off, cultures can be left for 5 days before reading. In fact, if formalin is added to keep down mould growth and evaporation is prevented by corking the tubes, they can be kept indefinitely for demonstration purposes.

(2) Since there is no gas lost by wheying off a much sharper differentiation is obtained between 'present' and 'much present', so a rather higher percentage of 'much present' results has been obtained. Frequently the curd is pushed up the tube right to the plug.

(3) No difficulty is experienced in reading tubes classed as 'trace', and although a small percentage of these may be 'false positives', duplicate readings by different workers agree well.

The results obtained may be compared with other published figures for the coliform content of butter. In England, Crossley (13) found eleven presumptive positives in seventy samples of imported salted butter, none of which was less than 6 months old. He also reported that Rice (14), working on 131 samples of freshly made Australian salted butter, found coliform germs present in sixty-one. Rice found, however, that a slow decline of the coliform content occurred during cool storage at 14° F. In the United States Parfitt (15), using sodium formate-ricinoleate broth, found 16·9% of presumptive positives in 1000 samples of commercial butter.

CLASSIFICATION OF COLIFORM TYPES

In order to differentiate the 257 organisms isolated as already described, and also to gain some idea of the distribution of coliform types in New Zealand butter, the usual further confirmatory tests were applied, as follows:

(1) Methyl red and Voges-Proskauer (O'Meara's modification) in dextrose phosphate broth.

(2) Growth in Koser's citrate medium.

(3) Indole production in peptone water.

(4) Liquefaction in gelatine stab cultures incubated for 1 month at 22° C., and if negative, followed by 1 month at 37° C.

One typical colony only was selected from each MacConkey plate and subcultured on a nutrient agar slope from which the tubes of confirmatory media were inoculated. When anomalous results (particularly those giving MR+, VP+) occurred, the cultures were purified by replating from a second MacConkey broth culture before being re-inoculated into the confirmatory media.

Table 4. *Classification of coliform cultures*

	MR	VP	Citrate	Indole	Gel. liq.	Total strains	Percentages
Coli Type I	+	−	−	+	−	76	30·8
Coli Type II	+	−	−	−	−	3	
Intermediate I	+	−	+	−	−	28	12·4
Intermediate II	+	−	+	+	−	4	
Aerogenes I	−	+	+	−	−	78	53·7
Aerogenes II	−	+	+	+	−	16	
Cloacae	−	+	+	−	+	44	
Irregular						8	3·1

The classification of coliform types, as summarized in Table 4, is similar to that used by Crossley (13), with the exception that, because of the smaller number of cultures tested, the irregular strains have not been differentiated.

In considering these results it must be stressed that only one colony was taken from each plate, although many plates appeared to have more than one type present. Apparently, however, there were significantly more organisms of the *aerogenes-cloacae* group isolated than of the faecal *coli* type. But as the investigation proceeded, what appeared to be a seasonal variation was noticed, as shown in Table 5.

Taking the warmer months to be April, May, October to January and the cooler, June to September, the faecal *coli* and also the intermediate types appeared to be present in larger numbers during the warmer months. (Unfortunately no differential tests were done during the two warmest months, February and March, when a still higher proportion of

Table 5. *Seasonal variation in coliform types*

	Warm months		Cold months	
Total number of samples	383		336	
	No.	Percentage	No.	Percentage
Coli (faecal) cultures	58	36·5	21	22·1
Intermediate cultures	27	17·0	9	9·5
Aerogenes-cloacae cultures	74	46·5	65	68·4

coli to *aerogenes* might have been found.) On the other hand, only a slight decrease occurred in the numbers of the *aerogenes-cloacae* group during the cold months. This variation, although the reverse of that reported by Malcolm (16) for milk in Scotland, may perhaps be due to the same cause. Malcolm suggested that the lower ratio of *coli* to *aerogenes* found in milk in the summer was due to the fact that the *aerogenes* strains and not the faecal *coli* could multiply rapidly at the temperatures commonly found in the milk (i.e. 17–22° C.), whereas winter temperatures (5–13° C.) were too low for even the *aerogenes* to multiply. Hence, as faecal *coli* predominate in bovine faeces, this type was also predominant in the milk during the winter. As regards the seasonal variation of the coliform organism in New Zealand butter it is suggested that the winter temperatures would favour the growth of the *aerogenes* but check faecal *coli* types. On the other hand, summer temperatures would frequently be high enough to encourage growth of faecal *coli* in pockets of milk residues in the factory equipment.

In contrast to the results obtained from New Zealand butter, other workers have found in general a higher proportion of the faecal types in butter. Thus quoting the respective figures for the *coli* and *aerogenes* strains only, Crossley (13) found a ratio of 46·4–43·9%; Rice (14) found 58·9–17·8%; and Parfitt (15) 59·6–32·1%. Some interesting comments on this subject have been made by Crossley.

ATYPICAL COLIFORM STRAINS

In the later stages of this investigation, sixteen atypical coliform organisms were isolated from presumptive positive renneted milk or litmus milk agar tubes. On MR–VP and gelatine liquefaction tests eleven were classed as *cloacae* strains, and three as *aerogenes*. None of these cultures was able to produce more than a trace of gas when inoculated, as a pure culture, into MacConkey broth, yet all could produce gas in milk. Possibly the bile salt in the MacConkey broth inhibits the fermentation of lactose by these strains; alternatively, the more abundant gas formation in the milk media may have been derived from traces of glucose or other sugar produced by hydrolysis. It is significant perhaps that of the thirteen doubtful positive MacConkey broth tubes recorded in Table 3, ten were *aerogenes-cloacae* strains. Barkworth & Irwin (17) found that bile salt broth has no bactericidal effect on coliform organisms, but presumably a faecal *coli* strain was used in their experiments. In view of the influence of various factors on gas production the results are recorded of a brief trial using another presumptive medium—lauryl sulphate tryptose broth (18). A series of 178 samples were cultured, each into MacConkey broth, litmus milk agar, and lauryl sulphate broth with tryptone instead of tryptose as specified. Gas formation was recorded for forty-five MacConkey cultures and forty-three milk agar, but for fifty-two in the lauryl sulphate broth. This last medium in fifteen cases showed more gas than the corresponding MacConkey tube.

Whether these atypical coliform strains are capable of playing any part in the production of flavour defects in butter is yet to be investigated, but their apparently close relationship to the *aerogenes-cloacae* group suggests that their presence in butter is as undesirable as any other member of the coliform group. Also the fact that 17% of the 257 organisms isolated were of the *cloacae* type suggests that some of the 'false positive' results obtained with the litmus milk cultures were perhaps due to the presence of bacteria of this type. Further investigations are desirable on the selective effect of litmus milk media and the depressing effect of bile salt lactose media on gas formation by the *aerogenes-cloacae* strains isolated from butter.

SUMMARY

1. The limitations have been explored of a simplified form of Grimes's litmus milk method, as used for a presumptive test for coliform organisms in butter.

2. To improve on this test, the merits and demerits of renneted litmus milk and litmus milk agar have been investigated, and the latter found preferable.

3. Of 719 samples of export salted butter tested for coliform contamination, only 19·1% gave confirmed positive results using MacConkey broth as the presumptive medium, 21·9% were positive using a litmus milk agar medium, and 30·2% (on 434 samples only) were positive using renneted milk.

4. The coliform organisms isolated were classified into the usual types and a seasonal variation in the *coli* to *aerogenes* ratio noted.

5. An observation is made on what appears to be the inhibitory effect of bile salt on the gas production of several strains of the *aerogenes-cloacae* group isolated in this investigation.

The author is indebted to Dr G. M. Moir for his interest in the work, and particularly for guidance in the preparation of the manuscript.

REFERENCES

(1) Pont, E. G. (1935). *J. Dairy Res.* **6**, 148.
(2) Wilson, G. S. (1935). *Spec. Rep. Ser. med. Res. Coun., Lond.*, no. 206.
(3) Grimes, M. (1934). *J. Dairy Sci.* **17**, 11.
(4) Hammer, B. W. & Yale, M. W. (1932). *J. Dairy Sci.* **15**, 199.
(5) Moir, G. M. & Russell, R. R. (1939). *J. Dairy Res.* **10**, 310.
(6) Hiscox, E. R. (1934). *J. Dairy Res.* **5**, 233.
(7) Oxley, C. D. (1938). *Proc. Soc. agric. Bact.*
(8) Burke-Gaffney, O. D. (1932). *J. Hyg., Camb.*, **32**, 85.
(9) Hassouna, M. M. & Allen, L. A. (1939). *J. Dairy Res.* **10**, 7.
(10) Orla-Jensen, S. (1931). *Dairy Bacteriology*, p. 181. London: J. & A. Churchill.
(11) Huttig, C. (1933). *Milchw. Ztg.*, **38**, nr. 58.
(12) Minster, J. T. (1932). *Analyst*, **57**, 615.
(13) Crossley, E. L. (1946). *J. Dairy Res.* **14**, 233.
(14) Rice, E. B. (1937). *Proc. Soc. agric. Bact.*
(15) Parfitt, E. H. (1936). *J. Dairy Sci.* **19**, 496.
(16) Malcolm, J. F. (1939). *J. Dairy Res.* **10**, 410.
(17) Barkworth, H. & Irwin, J. O. (1943). *J. Hyg., Camb.*, **43**, 129.
(18) Mallmann, W. L. & Darby, C. W. (1941). *Amer. J. Publ. Hlth*, **31**, 127.

(*MS. received for publication* 17 *October* 1949)

637·333·9:576.8

413. THE GROWTH REQUIREMENTS OF LACTOBACILLI IN RELATION TO CHEESE FLAVOUR DEVELOPMENT

By G. J. E. HUNTER
The Dairy Research Institute (N.Z.), Palmerston North, New Zealand

(With 1 Figure)

The development of flavour in Cheddar cheese is due mainly to the activities of lactobacilli which gain access to the cheese milk by chance contamination. Other factors, for instance lipase action, may contribute to flavour, but their precise role is at present undefined. It is well known that the bacteriological quality of milk has a large influence, for good or ill, on the flavour of cheese made from it, and that pasteurization of the cheese milk makes cheese flavour milder, more regular from day to day, and slower in developing. Is the mildness in flavour of pasteurized milk cheese due simply to the destruction of bacteria in the milk supply, or does the heat treatment bring about chemical changes in the milk which render the cheese curd less favourable as a medium for the bacteria which produce flavour? This question has not been settled, but Sherwood (1) showed that protein degradation takes a slightly different course in pasteurized as compared with raw milk cheese. Tittsler, Geib, Sanders, Walter, Sager & Lochry (2) and Sanders, Tittsler, Walter, Sager, Lochry & Geib (3) have shown, however, that raw milk containing few bacteria gives a cheese which ripens and develops flavour as slowly as a pasteurized milk cheese. It would seem, therefore, that an increase in the number of lactobacilli present in the cheese curd ought to hasten flavour development in a cheese, although there may also be some factor involving the suitability of the cheese curd as a medium which could influence the final result. Working at this Institute, Sherwood was able to show that the addition of lactobacilli to pasteurized cheese milk influenced the flavour (4) and the texture (5) of the final cheese. Different strains of lactobacilli imparted specific flavours (6). Some cultures produced desirable, and others undesirable, flavours. Attempts were made to improve cheese quality through the addition of selected cultures to pasteurized milk by incorporating the organisms in liquid rennet, or in starter cultures, or by addition to the milk of dried chalk-milk cultures (7). Of the three methods, the most convenient proved to be the starter culture method, but certain difficulties occurred, mainly because of the unsuitability of plain milk as a medium and the consequent need for an enrichment technique which could not have been carried out conveniently in commercial dairy factories.

A new approach to the problem was made recently. It was shown that when cheese curd was inoculated by spraying, in the final stages of the manufacturing process, with different strains of lactobacilli, the flavour of the cheese was altered. Two significant facts emerged: the first, that the effects, although quite definite in most cases, were much smaller than could be desired; the second, that a large inoculum gave little better results in the matured cheese than a much smaller inoculum. It seems clear then that other factors besides the presence of desirable types of lactobacilli are involved in the production of typical cheese flavour. It is of course well known that the character of a cheese depends to some extent on the nature of the cheese curd, especially upon the acidity of the green curd.

A very 'sweet' curd results in 'yeasty' fermentation flavours. A slightly 'sweet' curd seems to give finally in the mature cheese the most desirable flavours. A somewhat 'acid' curd may result in the development of a bitter flavour, while a very acid cheese is 'sharp' in taste and is generally bleached in colour. There is little doubt, therefore, that the condition of the curd as a medium for bacterial growth may determine which type of flavour-producing organism shall be encouraged to grow during the ripening period. Thus, in order to control and promote the development of a desirable flavour in maturing cheese, probably at least three requirements must be satisfied.

(i) The desirable types of lactobacilli must be present.

(ii) There must be a minimum of undesirable organisms present.

(iii) Conditions must be as favourable as possible for the growth of the desirable types.

The object of the present paper is to report preliminary results obtained from laboratory studies of the growth requirements of various strains of lactobacilli under conditions similar to those obtaining in cheese. With the knowledge gained, it is hoped that addition of lactobacilli and/or the adjustment of conditions in the cheese curd so as to favour certain types may ultimately lead to greater control of cheese flavour than has hitherto been possible.

Preparation of basal medium

It was first necessary to devise a medium containing water-soluble products similar to those which may occur in the moisture of cheddar cheese. This seemed a logical step in any endeavour to ascertain the growth characteristics of the different strains of lactobacilli in cheese, a medium which differs in so many respects from artificial media commonly used in the laboratory. Preliminary experimentation led to the formulation of a medium based on the soluble products formed by digestion of casein by rennet. The casein was prepared from fresh skim milk (warmed to 34·5° C.) by precipitation at pH 4·7 with 10% lactic acid. After removal of the acid whey, the product was washed four times in distilled water, with gentle mechanical stirring, for a period of 15–20 min. To the moist washed casein a measured quantity of commercial rennet was added. The mixture, contained in a well-stoppered bottle and saturated with chloroform, was held at 30° C. for 7 days with occasional shaking and stirring. At the end of this incubation period a volume of distilled water equal to the weight of the original moist casein was added with careful warming, and the soluble breakdown products were filtered off. The chloroform was removed by steaming. After a few trials a standardized procedure was adopted on the following lines:

Fresh skim milk	3 l.
Lactic acid 10%	175 ml.
Moist washed casein obtained	300 g.
Rennet added	15 ml. commercial rennet diluted with equal volume distilled water.
Chloroform	10 ml.
Incubation	7 days at 30° C.
Distilled water added	300 ml. mixture heated to near boiling-point and filtered.
Volume of filtrate	400 ml. (approx.).

The resultant solution (hereafter referred to as casein digest) served as the basal nitrogen source in the medium. The various final media were tubed in 10 ml. quantities and, after

sterilization, were inoculated with one drop (from a pasteur pipette) of a 1 in 100 dilution of a milk culture of the lactobacillus under investigation. The organisms used were those isolated by Sherwood (6) from New Zealand cheddar cheese. The strain numbers given in the tables are those used by Sherwood. The incubation temperature was 30° C. Extent of growth was estimated by examination of stained smears under the microscope. The following general conclusions were drawn from observations on the growth of a selection of strains of lactobacilli in the casein digest alone and in various media prepared from it.

(1) *The influence of rennet*

Media prepared from aqueous extracts of washed casein without rennet treatment did not support active growth of the lactobacilli. Protein breakdown products derived from rennet digestion of the moist casein were found to be essential for good growth of all the organisms, and all subsequent experiments were therefore carried out in media prepared with a basis of casein digest. The rate and extent of growth of the organisms in the final medium varied with the proportion of rennet used and with the time of contact of the rennet with the casein at 30° C. before preparation of the medium. Five per cent of rennet (the maximum proportion used) and incubation for 7 days gave the best results. A higher proportion of rennet could not readily be used because commercial rennet contains about 19% of salt which had to be kept below an inhibitory level in the final medium.

(2) *The effect of washing the precipitated casein*

All our strains of lactobacilli gave good growth (at least +++) within 2 days, increasing to heavy growth (++++) within 7 days when inoculated into media prepared by the action of rennet on casein which had been washed only once. As will be seen from Table 1, four washes resulted in much poorer growth of the organisms, while six washes had the effect of reducing growth to traces even after 14 days' incubation. The growth-promoting power of the medium could be almost fully restored by the addition of lactose, which is probably the main substance removed by washing. Some of the later results suggested, however, that other water-soluble growth factors may have been removed from the casein curd during its preparation. For all subsequent work a standard procedure employing four washes was followed.

(3) *The effect of the* pH *of the medium*

A pH range of 5·1–5·6 in the final medium proved optimum for growth of all the strains of lactobacilli examined.

(4) *The effect of various additions to the casein digest*

(*a*) *Carbohydrates.* The effect of added carbohydrate was studied in media prepared from well-washed casein as well as from sugar-free casein (Merck) after the usual digestion with rennet. The addition of lactose to the casein digest improved growth considerably (Table 1). As might be expected, the effect of other sugars varied with individual cultures, but it was evident that fermentable carbohydrate was necessary for the rapid proliferation of the lactobacilli.

(*b*) *Lactates.* Sodium lactate did not assist growth when it was added to the casein digest medium. With some strains of lactobacilli a concentration of 1% was slightly inhibitory. Its main effect, however, was that of a buffer and in its presence a much stronger

growth occurred in sugar-containing media before the pH dropped to suboptimal levels. Growth of the lactobacilli ceased at pH 4·0. Lactate was included in the final medium because it is normally present in cheese curd. The lactate was prepared from 10% lactic acid by neutralization with caustic soda to a pH of approximately 5·8. After boiling or steaming the pH was 5·4.

(*c*) *Mineral salts.* An addition of phosphate to the medium was definitely beneficial for growth. A further addition of a mixture containing Ca, Mg, Fe, Mn and K ions gave increased growth. It was evident that there was a lack of some at least of these elements in the final medium possibly as a result of the washing of the casein. Some light was thrown on this point in the later experiments.

(*d*) *Nitrogenous materials.* The basal casein digest contained 0·44% of nitrogen or 2·8% of protein. Additional nitrogen sources such as commercial peptones, and mixtures of amino-acids were without effect on the growth-promoting properties of the medium.

(*e*) *Vitamins.* Additions of riboflavin and other known members of the vitamin B complex did not stimulate growth.

At this stage in the investigation, the following medium was adopted as being the one giving as good a growth as possible of the lactobacilli, within the limits imposed by the requirement that the nutrients in the medium should be similar to those in cheese:

Casein digest	Prepared as previously described
Lactose	0·5%
Na_2HPO_4 (anhyd.)	0·1%
Sodium lactate solution	1%
pH	5·4 (no adjustment needed)

Although the medium supported a fair growth of all the strains of lactobacilli under examination, the normal yeast-milk medium used in the laboratory gave a better growth, and it was evident that the casein digest medium was lacking in some growth-promoting substance or substances. Do such substances occur in cheese or is the growth of lactobacilli limited in cheese just as it is in the casein digest medium?

Minerals as growth stimulants for the lactobacilli

A chance suggestion was made that cheese whey which had been concentrated during the commercial extraction of lactose might contain materials which would improve the presumably deficient medium. Trial showed that whey paste (mother liquor), obtained after extraction of the bulk of the lactose from cheese whey, gave a marked stimulation of the growth of lactobacilli when added in small amounts to the casein digest medium. The whey paste had the following gross composition:

Moisture	57·9%	Lactose	17·5%
Protein	13·1%	Ash	8·1%

It was found that none of the nitrogenous constituents nor the lactose was responsible for the stimulating action. The paste was shown by microbiological assay to contain significant amounts of thiamine, riboflavin, pantothenic acid, nicotinic acid, pyridoxine, biotin, *p*-aminobenzoic and folic acids, but none of these substances gave a significant effect when added to the casein digest medium. Finally, it was found that the ash left after ignition of the whey paste contained all the growth-stimulating material, and that a watery extract

Table 1. *The effect of washing casein during preparation of a medium for lactobacilli*

(Medium: casein digest (prepared by the action of rennet on casein which had been washed one, four and six times) containing 0·1% Na_2HPO_4, pH 5·3.)

Organism and strain no.	One wash		One wash + 0·5% lactose		Four washes		Four washes + 0·5% lactose		Six washes			Six washes + 0·5% lactose		
	Time of incubation of cultures (days)													
	2	7	2	7	2	7	2	7	2	7	14	2	7	14
3.1 *Pc*	+ + +	+ + + +	+ + +	+ + + +	+ +	+ + +	+ + + +	+ + + +	–	–	–	+ + + +	+ + + +	+ + + +
1.1 *Pc*	+ + + +	+ + + +	+ + + +	+ + + +	+ +	+ + +	+ + + +	+ + + +	Tr.	Tr.	Tr.	+ +	+ + +	+ + + +
6.2 *Pc*	+ + + +	+ + + +	+ + + +	+ + + +	+	+ +	+ + + +	+ + + +	–	–	–	+ + + +	+ + + +	+ + + +
4.3 *Pc*	+ + +	+ + + +	+ + +	+ + + +	+	+ + +	+ + + +	+ + + +	–	–	Tr.	+ + +	+ + + +	+ + + +
10.5 *Pb*	+ + + +	+ + + +	+ + + +	+ + + +	+	+	+ + + +	+ + + +	–	–	Tr.	+ + + +	+ + + +	+ + + +
27.1 *Cb*	+ + + +	+ + + +	+ + + +	+ + + +	+ +	+ + +	+ + + +	+ + + +	–	Tr.	Tr.	+ +	+ + +	+ + + +

Classification: *P* = *Sbm. plantarum*; *C* = *Sbm. casei*; *c* = variety C; *b* = variety B.

Table 2. *Growth-stimulating property of concentrated whey paste*

(Medium: casein digest containing 0·5% lactose, 0·1% Na_2HPO_4, 1% Na lactate, pH 5·4.)

Organism and strain no.	Control			+0·1% Whey concentrate			+Water-soluble extract			+Water-soluble ash			+Acid-soluble ash		
	Time of incubation of cultures (days)														
	2	7	14	2	7	14	2	7	14	2	7	14	2	7	14
10.2 *Pa*	–	–	Tr.	Tr.	+	+	Tr.	+	+ +	–	Tr.	+	–	Tr.	+
10.5 *Pb*	Tr.	+	+ +	+ +	+ + + +	+ + + +	+	+ + +	+ + + +	Tr.	+ + +	+ + +	+	+ + +	+ + + +
3.1 *Pc*	–	+	+ +	+	+ + +	+ + +	Tr.	+ +	+ + +	Tr.	+ +	+ + +	Tr.	+ +	+ + +
1.1 *Pc*	–	+	+ +	+	+ + + +	+ + + +	+	+ +	+ + + +	+	+ +	+ + +	+	+ + +	+ + +
6.2 *Pc*	Tr.	+	+ +	+ +	+ + + +	+ + + +	+ +	+ + +	+ + + +	+	+ + +	+ + +	+	+ +	+ + + +
4.3 *Pc*	Tr.	+	+ +	+ +	+ + + +	+ + + +	+	+ + +	+ + + +	+	+ + +	+ + +	+	+ +	+ + + +

P = *Sbm. plantarum*; *a* = variety A; *b* = variety B; *c* = variety C.

of this ash added to the medium in an amount corresponding to 0·005 g. of ash per ml. of medium gave substantially the same effect as an addition of the original whey paste. The results obtained by additions of whey paste, water-soluble extract, and water and acid-soluble extracts of ash are given in Table 2.

It was clear from these results that the casein digest medium could be improved by addition of some mineral salts, which may have been removed from the casein in the washing process during preparation of the medium. A consideration of the possibilities led first to a trial of the effect of potassium.

Influence of potassium on the growth of lactobacilli

Of the mineral constituents of milk potassium is known to be essential for the growth of all lactobacilli. Analysis of the whey paste showed the presence of 1·95% or 19·5 mg. K per g. of paste. When added to the medium, all potassium salts gave marked stimulation of growth of the organisms, provided of course that the accompanying anion was not toxic in the concentration used. The corresponding sodium salts did not act as stimulants (Table 3). In the casein digest medium a concentration of added potassium ions of 0·02 mg./ml. was required to give significant stimulation of growth. The concentration required for maximum stimulation under the conditions of the trials was 0·1 mg./ml. (Table 4).

A paper of particular interest dealing with the influence of other ions on the K ion requirements of lactic acid bacteria was recently published by MacLeod & Snell (8). They found that the amount of K usually required for maximum growth was high, and that the quantitative K requirement was markedly influenced by the presence of Na and NH_4 ions in the medium. As the concentration of Na ions rose to an inhibitory level the concentration of K ions required to prevent inhibition also increased. They showed that the toxic action of Na ions is due to interference with utilization of K and is prevented by addition of K ions.

Because of the possible importance of such 'ion antagonism' in the present investigation, the phenomenon was examined using the high percentages of NaCl necessary in cheese making. The results are given in Table 5. The casein digest medium already contained a high concentration of Na ions (approximately 5 mg./ml.) derived from the sodium phosphate and sodium lactate and from the salt in the rennet. It will be seen from the results in Table 5 that in accordance with the findings of MacLeod & Snell, the addition of K ions (in the form of KCl) in concentrations of 0·01–0·05 mg./ml. of medium was sufficient to overcome the inhibitory action of even 4% of added NaCl in some instances. The greater the proportion of K added, the earlier was the initiation of growth.

Influence of manganese ions

Demeter (9) and Orla-Jensen, Orla-Jensen & Snog-Kjaer (10) recently reported that streptobacteria, in contrast to streptococci, are stimulated by traces of manganese in the medium and that the optimum concentration in milk for these organisms is 10 mg. of $MnCl_2$, $4H_2O$ per litre. The ash of the whey paste used in the present experiments did not contain significant amounts of manganese, but addition of manganese salts to the casein digest medium proved stimulating to the growth of some strains of lactobacilli. Tables 6 and 7 illustrate the results obtained, and indicate how very small proportions of manganese

Table 3. *Effect of potassium and sodium salts on the growth of lactobacilli*

(Medium: casein digest containing 0·5% lactose, 0·1% Na_2HPO_4, 1% Na lactate, pH 5·4.)

Organism and strain no.	Control			+0·1% KNO_3			+0·1% K_2SO_4			+0·1% $KClO_3$			+0·1% KHPhthalate			+0·1% $NaNO_3$			+0·1% $NaNO_2$			+0·1% Na_2SO_4		
	Time of incubation of cultures (days)																							
	2	7	14	2	7	14	2	7	14	2	7	14	2	7	14	2	7	14	2	7	14	2	7	14
10.2 *Pa*	–	–	Tr.	–	Tr.	Tr.	–	Tr.	Tr.	–	Tr.	Tr.	–	Tr.	Tr.	–	–	–	–	–	–	–	–	Tr.
10.5 *Pb*	Tr.	+	++	+	+++	+++	+	+++	+++	+	+	+++	+	+++	+++	Tr.	+	++	Tr.	Tr.	+	Tr.	+	++
3.1 *Pc*	–	+	++	+	++	+++	+	++	+++	+	+	++	Tr.	++	+++	–	+	++	–	Tr.	+	–	+	++
1.1 *Pc*	–	+	++	++	+++	++++	++	+++	+++	++	+++	+++	++	+++	++++	–	+	++	–	Tr.	Tr.	–	+	++
6.2 *Pc*	Tr.	+	+	++	+++	+++	++	+++	+++	++	++	+++	+	++	+++	Tr.	++	++	–	Tr.	+	Tr.	+	+

P = *Sbm. plantarum*; *a* = variety A; *b* = variety B; *c* = variety C.

Table 4. *Effect of different concentrations of potassium on the growth of lactobacilli*

(Medium: casein digest containing 0·5% lactose, 0·1% Na_2HPO_4, 1% Na lactate, pH 5·4; potassium added as KCl salt.)

Organism and strain no.	mg./ml. K^+ added: Nil			0·01			0·02			0·1			1·0		
	Time of incubation (days)														
	2	7	14	2	7	14	2	7	14	2	7	14	2	7	14
10.2 *Pa*	–	–	Tr.	Tr.	Tr.	+	Tr.	Tr.	+	Tr.	Tr.	+	Tr.	Tr.	+
10.5 *Pb*	Tr.	+	+++	+	+	++++	+	+++	++++	+	+++	++++	+	+++	++++
3.1 *Pc*	–	++	+++	–	++	+++	Tr.	++	++++	+	++	++++	+	++	++++
1.1 *Pc*	–	++	+++	Tr.	++	+++	Tr.	++	++++	+	+++	++++	+	+++	++++
6.2 *Pc*	Tr.	++	+++	+	++	+++	+	++	++++	+	++	++++	+	++	++++

P = *Sbm. plantarum*; *a* = variety A; *b* = variety B; *c* = variety C.

Table 5. *Effect of addition of* K *ions to medium containing varying amounts of* NaCl

(Medium: casein digest containing 0·5% lactose, 0·1% Na_2HPO_4, 1% Na lactate, pH 5·4. KCl added to give required concentration of K ions per ml. medium.)

Organism and strain no.	Medium	Added NaCl, nil		Added NaCl, 1%		Added NaCl, 2%		Added NaCl, 3%	
		Time of incubation (days)							
		2	7	2	7	2	7	2	7
3.1 *Pc*	Control	–	+	–	++	–	+	–	–
	+0·01 mg. K^+/ml.	Tr.	++	Tr.	++	–	++	–	+
	+0·05	+	+++	+	+++	Tr.	+++	–	+++
	+0·1	++	+++	++	+++	Tr.	+++	–	++
	+0·5	++	+++	++	+++	++	+++	++	+++
1.1 *Pc*	Control	–	++	–	++	–	–	–	–
	+0·01 mg. K^+/ml.	–	++	–	++	–	+	–	–
	+0·05	Tr.	++	–	+++	–	+++	–	Tr.
	+0·1	+	+++	Tr.	+++	–	+++	–	++
	+0·5	++	+++	++	+++	+	+++	+	+++

Organism and strain no.	Medium	Added NaCl, nil		Added NaCl, 2%		Added NaCl, 3%		Added NaCl, 4%	
		Time of incubation (days)							
		2	7	2	7	2	7	2	7
21.1 *Pd*	Control	–	+++	–	Tr.	–	–	–	–
	+0·05 mg. K^+/ml.	++	+++	Tr.	+++	–	++	–	–
	+0·1	++	++++	+	++++	Tr.	+++	–	++
	+0·5	+++	++++	+++	++++	+++	++++	++	+++
	+1·0	+++	++++	+++	++++	+++	++++	+++	++++
1.2 *Bbm*	Control	+	+++	–	–	–	–	–	–
	+0·05 mg. K^+/ml.	++	+++	+	++	–	++	–	–
	+0·1	++	+++	++	+++	Tr.	+++	–	Tr.
	+0·5	+++	+++	+++	+++	++	+++	–	+
	+1·0	+++	+++	+++	+++	++	+++	Tr.	++

P = *Sbm. plantarum*; *c* = variety C; *d* = variety D; *Bbm* = Betabacterium.

Table 6. *Effect of manganese on the growth of lactobacilli in media deficient and rich in potassium*

(Basal medium: casein digest containing 0·5% lactose, 0·1% Na_2HPO_4 or K_2HPO_4, 1% Na lactate or K lactate, pH 5·4. Mn^{++} added as $MnSO_4$.)

Organism and strain no.	Medium	Casein digest +lactose +Na_2HPO_4		Casein digest +lactose +Na_2HPO_4 +Na lactate		Casein digest +lactose +K_2HPO_4		Casein digest +lactose +K_2HPO_4 +K lactate	
		Time of incubation (days)							
		2	7	2	7	2	7	2	7
3.1 *Pc*	Control	+	+ + +	–	+ +	+ + +	+ + + +	+	+ + + +
	+0·1 mg. Mn^{++}/ml.	+	+ + + +	–	+ + + +	+ + + +	+ + + + +	+ + +	+ + + +
	+0·5 mg. Mn^{++}/ml.	+	+ + + +	–	+ + + +	+ + + +	+ + + + +	+ + +	+ + + + +
	+1·0 mg. Mn^{++}/ml.	+ +	+ + + +	–	+ + + +	+ + + +	+ + + + +	+ + +	+ + + + +
1.1 *Pc*	Control	+	+ + + +	–	+ + + +	+ +	+ + + +	+ +	+ + + +
	+0·1 mg. Mn^{++}/ml.	+	+ + + + +	–	+ + + + +	+ + + +	+ + + + +	+ + +	+ + + + +
	+0·5 mg. Mn^{++}/ml.	+ +	+ + + + +	–	+ + + + +	+ + + +	+ + + + +	+ + + +	+ + + + +
	+1·0 mg. Mn^{++}/ml.	+ +	+ + + + +	–	+ + + + +	+ + + +	+ + + + +	+ + + +	+ + + + +

Table 7. *Effect of manganese on growth of lactobacilli*

(Basal medium: casein digest containing 0·5% lactose, 0·1% K_2HPO_4, 1% K lactate; pH 5·4. Mn added as $MnSO_4$.)

Organism and strain no.	mg./ml. of Mn^{++} added: Nil		0·00001		0·0001		0·001		0·01		0·10	
	Time of incubation (days)											
	2	7	2	7	2	7	2	7	2	7	2	7
10.2 *Pa*	–	Tr.	–	–	–	–	–	Tr.	–	Tr.	–	Tr.
10.5 *Pb*	Tr.	+ +	+ +	+ + +	+ + +	+ + +	+ +	+ + +	+ +	+ + + +	+ + +	+ + + +
3.1 *Pc*	Tr.	+	+ +	+ + +	+ + +	+ + + +	+	+ + +	+ +	+ + + +	+	+ + + +
1.1 *Pc*	+	+ + +	+	+ + +	+	+ + +	+	+ + +	+ +	+ + +	+ + +	+ + + +
6.2 *Pc*	+	+ + +	–	–	–	–	+ +	+ + + +	+ +	+ + + +	+ + +	+ + + +

show a significant effect. Whereas K ions seem mainly to promote early initiation of growth, Mn ions give increased growth as compared with the control throughout the growth period of 14 days.

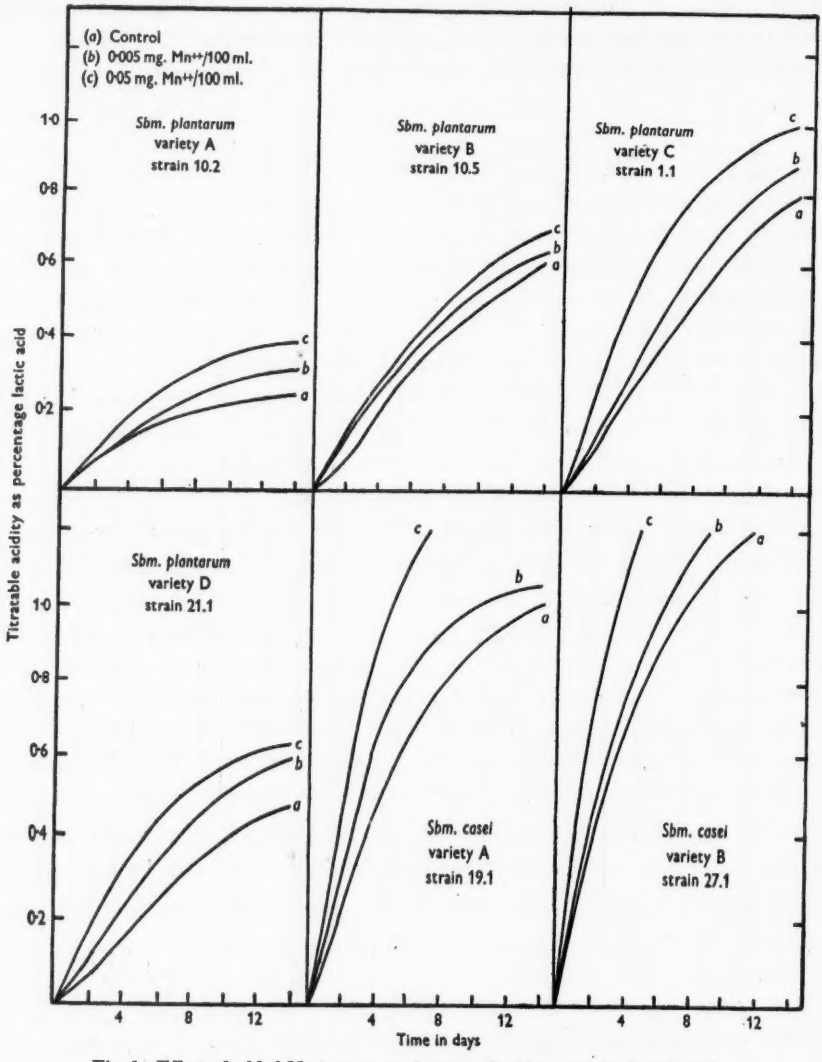


Fig. 1. Effect of added Mn ions on production of acid in milk by lactobacilli.

The effect of added potassium and manganese in milk

The casein digest medium employed throughout this work was designed to stimulate cheese as a medium for the lactobacilli. The medium in its earlier forms was evidently deficient in certain growth factors, as cheese itself may be, and thus the growth-promoting effect of certain substances was more readily demonstrated than in a medium like milk. Trials with milk as a medium showed that the lactobacilli did not respond to additions of K ions, presumably because milk already contains about 100 mg. of K per 100 ml., which is ample for growth requirements. Further it was not possible, for the same reason, to demonstrate clearly in milk the antagonism between Na and K ions. The stimulating

action of Mn ions was, however, quite evident in milk. Manganese is present in normal milk only in minute proportions of the order of 0·003 mg./100 ml. The addition of as little as 0·001 mg. of Mn^{++} per 100 ml. was found to have a significant effect on the growth of lactobacilli as judged by their acid production. The graphs in Fig. 1 illustrate the effect of the additions of Mn ions to milk on the growth of various strains of lactobacilli. It is evident that the stimulating effect varies considerably from strain to strain.

DISCUSSION

The finding that K and Mn ions play a significant part in the growth of the lactobacilli which occur in cheddar cheese is very interesting in any consideration of the mechanism of cheese flavour development. There appears to be a distinct possibility that whereas milk contains enough potassium to serve the needs of the bacteria, cheese curd may be deficient in potassium. There may well be variation in the potassium content of curd according to the particular milk used for cheese manufacture. Little is known, however, of the distribution of potassium or the trace elements between cheese curd and cheese whey. Another factor which enters into the situation in cheese curd is the balance which exists between potassium and sodium ions. The addition of salt to curd (usually enough to give a concentration of 3·5–4·5% in the cheese moisture 14 days after manufacture) tends to have an inhibitory effect on the growth of the lactobacilli. An increased proportion of potassium ions would tend to offset this effect. The role of manganese in stimulating the growth of lactobacilli falls into quite a different category because this element does not normally occur in milk in amounts necessary for optimal growth of the organisms.

One has a natural tendency to assume that cheese curd is a medium peculiarly suitable for the growth of lactobacilli, because these organisms usually assume a dominant role in the flora of ripening cheese. The present results suggest, however, that conditions in cheese may not particularly suit the lactobacilli, although evidently they suit other bacteria still less. If this is so the enhancement of flavour in curd produced from a milk of low bacterial count, pasteurized before cheese manufacture begins, becomes quite a new problem, not merely an attempt to copy the conditions which obtain where a poor quality milk containing large numbers of a variety of types of bacteria is used. Under the former conditions the development of a desirable flavour in a short time may involve the addition of elements, such as manganese, which do not normally occur in any type of cheese curd. In other words, it may be a matter of rendering cheese curd made from high-quality pasteurized milk a more favourable medium for lactobacilli than it ever is under normal conditions. It is inevitable that lactobacilli in cheese curd should be short of carbohydrate because the lactose in the cheese is rapidly fermented in the first few weeks of the ripening period by the lactic streptococci. The lactobacilli apparently cannot use lactate as a growth substance, although it may serve a useful purpose as a buffer. In an attempt to determine whether carbohydrate shortage is a limiting factor for the lactobacilli in cheese curd, some preliminary experiments were carried out in which carbohydrates fermentable by certain strains of lactobacilli but not by lactic streptococci were added to cheese curd along with the salt. A portion of this curd was then sprayed with a culture of the appropriate strain of flavour-producing lactobacilli. The flavour of the experimental cheese gave no evidence of an enhanced growth of lactobacilli, and it was tentatively concluded that carbohydrate shortage is not the main limiting factor in the growth of lactobacilli in cheese.

The implications of these laboratory results need to be tried in cheese manufacturing

practice, and it is intended to follow up the possibility that additional potassium and manganese may stimulate the growth of lactobacilli in cheese, and hence may enhance and speed the development of cheese flavour. Early results indicate that potassium has a definite effect on cheese flavour, but a considerable amount of work is necessary before the practical significance of the effect can be assessed.

SUMMARY

An attempt was made to devise a medium for the growth of lactobacilli which would simulate the nutritive conditions existing in ripening cheddar cheese. The basal medium finally used contained soluble digestion products of casein (formed by the action of rennet), lactose, lactate and phosphate. This medium gave a fair, but not a very heavy growth of flavour-producing lactobacilli isolated from cheddar cheese, and it was evidently deficient in growth-promoting power as compared with some media used in the laboratory. Additions of potassium and manganese salts to the casein digest medium gave a significant increase in growth. Potassium had the effect of off-setting the inhibition caused by the presence of an excess of sodium salts such as are normally present in cheese. The implications of the results in cheese ripening and their possible application in cheese manufacture are discussed.

The author is indebted to Dr H. R. Whitehead for helpful criticism, and to Mr A. Holmes, of Dairy Products Ltd., Kapuni, Taranaki, for a supply of whey paste.

REFERENCES

(1) Sherwood, I. R. (1936). *J. Dairy Res.* **7**, 271.
(2) Tittsler, R. P., Geib, D. S., Sanders, G. P., Walter, H. E., Sager, O. S. & Lochry, H. R. (1946). *J. Bact.* **51**, 590.
(3) Sanders, G. P., Tittsler, R. P., Walter, H. E., Sager, O. S., Lochry, H. R. & Geib, D. S. (1946). *Milk Pl. Mon.* **35**, 43.
(4) Sherwood, I. R. (1937). *J. Dairy Res.* **8**, 224.
(5) Sherwood, I. R. (1939). *J. Dairy Res.* **10**, 326.
(6) Sherwood, I. R. (1939). *J. Dairy Res.* **10**, 426.
(7) Sherwood, I. R. (1939). *J. Dairy Res.* **10**, 449.
(8) MacLeod, R. A. & Snell, E. E. (1948). *J. biol. Chem.* **176**, 39.
(9) Demeter, K. J. (1949). *Milchwissenschaft*, **4**, 97.
(10) Orla-Jensen, S., Orla-Jensen, A. D. & Snog-Kjaer, A. (1948). *K. Dansk. vidensk. Selsk, Biol. Skr.*, **6**, no. 1, 1–14.

(*MS. received for publication* 19 *October* 1949)

637.128:616.9

REVIEWS OF THE PROGRESS OF DAIRY SCIENCE

SECTION F. MILK-BORNE DISEASES

SAFE MILK. THE GENERAL POLICY IN GREAT BRITAIN

The general policy as regards the provision of a safe, and at the same time adequate, milk supply in this country is about to enter a new phase. The contending interests of producer and consumer will now become merged, and, in the near future, the progress which is envisaged in the new legislation sponsored by the Government will become an accomplished fact. During the past few years the medical press and parliamentarians in both Houses have stressed the urgency of the need for new legislation. In the House of Lords, Lord Bledisloe's recommendations as quoted in a leading article in the *Lancet*(1) were as follows: (1) a proper survey of the incidence of cattle disease throughout the country; (2) increasing the bonus given for milk from attested herds, and an intimation that, within a fixed period, milk from herds which are not free from tuberculosis will not be accepted for human consumption; (3) establishing a free State veterinary service; (4) marking cows eliminated from attested herds so that they cannot be sold into other herds and so spread disease without the purchaser knowing that the animals are affected; (5) compulsory vaccination of calves against contagious abortion; (6) compulsory health service for cattle; (7) establishing disease-free areas; and (8) a State abattoir service under veterinary supervision. During the debate it was stated that the annual milk supply was reduced by over 200 million gallons owing to disease. The editorial in the *Lancet* also pointed out that although the recommendations of Lord Bledisloe were admirable the needs of the people were immediate, and since all admitted that milk is being contaminated to a greater or less degree and is unsafe, immediate steps should be taken to rectify this by pasteurization. As Prof. G. S. Wilson pointed out in his book, *The Pasteurization of Milk*(2), 'Accredited milk is just as heavily infected with tubercle bacilli as ungraded milk; and Tuberculin Tested milk is just as heavily infected with *Br. abortus* as ungraded milk, if not more so.'

In 1945, also, an editorial in the *British Medical Journal*(3), entitled 'Milk still unsafe', dealt with a debate which had taken place at the Annual Representative Meeting of the British Medical Association. It was emphasized that, so far, nothing had been done to implement the recommendation in the Government's White Paper on 'Measures to improve the quality of the Nation's Milk Supply'(4), particularly in regard to the fact that the Minister of Food was enabled by a Defence Regulation (55G) to make it an offence to sell milk in any area which he might schedule, unless the milk was either heat-treated or from disease-free animals. The editorial also pointed out that American troops in this country had not been allowed to drink milk unless obtained from a tuberculin-tested herd and then pasteurized. In 1946, as reported in a further editorial in the same journal(5), the House of Lords again raised the question of the safety of the milk supply, and Lord Rothschild 'rose to call the attention of His Majesty's Government to the urgent need for compulsory pasteurization of milk in as many parts of the United Kingdom as is practicable'. He also pointed out that such diseases as undulant fever, enteric fever, dysentery, food poisoning, scarlet fever and diphtheria were still being transmitted through the agency of milk, and that so far as tuberculosis was concerned 1600 deaths and 7000 to 8000 active infections were caused annually by drinking infected milk. Lord De La Warr advocated cleaning up the herds and farms of producer retailers, while in the same debate Lord Ammon said that in England and Wales about 1075 million gallons of liquid milk were consumed annually and of these 725 million gallons were already heat-treated.

Chalmers(6) gave a review of the milk situation in Scotland, and showed that marked progress had been made in recent years towards the production and distribution of a safe and clean milk. In the year 1937, the number of attested herds in Scotland was 769, whereas in 1945 it was 3762. Under the Milk-in-Schools Scheme just over 500,000 children or 68% of those in the register of

all schools, under education authorities, were consuming 450,000 gallons per month, thus showing the necessity for safe milk. Again in Report No. 1 (7) of an Investigation into the Economics of Milk Production in Scotland, a foreword states that out of 8000 farms nearly one in four sells milk regularly throughout the year, and, on most of these, milk is the predominant enterprise and source of income. Milk output from all Scottish farms was valued at £22 millions in 1946/7 and constituted 28% of the total value of farm output, or rather more than the value of all crop sales. These figures are clear evidence of the importance of milk production in the Scottish farm economy. The value of milk to the consumer, and its unique place in the national diet, need no emphasis.

In the Report of the Committee (English) on Milk Distribution (8), the recommendations on the production of safe milk were as follows: (*a*) As an ultimate objective compulsory heat-treatment should be applied to all milk sold for liquid consumption, except milk sold in remote and thinly populated areas. (*b*) Until compulsory heat-treatment can be applied generally, raw T.T. milk should be permitted to be sold in any area. (*c*) The sale of accredited milk (standard milk in Scotland) should be prohibited from the outset in any area scheduled for compulsory heat-treatment. (*d*) In scheduled areas (i) persons in depots and dairy premises handling milk until it is bottled should be regularly examined for infections which can be milk-borne; (ii) tests of milk passing through such premises should be taken at least once every 2 weeks; (iii) the practice of selling loose milk should be prohibited and heat-treated milk should be filled into the containers in which it is to be delivered to the consumer; (iv) milk containers and their sealing should conform to standards no less stringent than those laid down for T.T. milk. (*e*) The description 'heat-treated' should be abolished. (*f*) The Central Authority should provide or contribute towards funds for research into problems connected with the handling and treatment of milk. (*g*) A training scheme for dairy technicians should be instituted.

On 23 November 1948, the Government formally introduced the Milk (Special Designations) Bill in the House of Lords. Under this, the Minister of Food is taking powers to enable him progressively to require all milk sold to conform to the standard of the best, and this will be done by an extension of pasteurization. The approved specially designated milks in England and Wales will be T.T., (Certified) T.T., Accredited, derived from a single herd, Pasteurized and Sterilized milk. In Scotland there will be Certified T.T., Standard, derived from a single herd, Pasteurized and Sterilized milk. Recognition of accredited, standard, and specially designated milks will be restricted to a period of 5 years from the commencement of the Act, and farmers who produced them now would thus be given a reasonable chance to up-grade their herds. When the Bill was read a second time in the House of Commons, the Minister of Food said that before looking to the question whether not to pasteurize T.T. milk the Ministry should concentrate on making safe by pasteurization the 30% of milk which was undoubtedly unsafe. That was the purpose of the Bill. The only harmful action which pasteurization had was to reduce the vitamin C content slightly. Under the Bill the powers to enforce cleanliness on farms would pass from the Local Authorities to the Ministry of Agriculture. The Minister of Food concluded by stating that the Bill hastened slowly, and that 5 years was a long time to tolerate the consequences of the sale of unsafe milk but, at all events, a start had been made to rid the country of what had been in the nature of a national disgrace. The introduction of the Bill has already had a considerable effect, for many dairy farmers are in process of arranging for their herds to be converted to T.T.

Finally, in the Report of the Committee on Milk Services, Scotland (9), the present arrangements for the control and distribution of milk supplies were reviewed and criticized, and various recommendations were made for an improved system of control. Chief among these were the following: (1) That the Department of Agriculture for Scotland should be made responsible for all aspects of milk production up to the stage of delivery to the first buyer, and that the powers and duties of Public Health Authorities under the Milk and Dairies legislation and Milk (Special Designations) Orders with regard to milk production should be transferred to the said Department, but subject to the Public Health Authorities retaining their powers and duties in relation to milk-borne diseases and diseases affecting dairy personnel and also their ordinary powers and duties in relation to public health. (2) That the Veterinary Service of the Animal Health Division should be transferred to the Department of Agriculture for Scotland from the Ministry of Agriculture and

Fisheries. (3) That animals of exempted producers should be made subject to regular veterinary inspection by the Animal Health Division and that such producers should, as far as practicable, ultimately be included in any national milk-testing scheme. (4) That the designated milks should eventually be reduced to not more than three: 'Certified', 'Tuberculin-tested' and 'Pasteurized', and that steps should be taken to familiarize the public with the significance of these designations. (5) (i) That areas largely free from bovine tuberculosis should be scheduled, and cleared of reactors after 2 years; (ii) that vaccination of young females in dairy herds against contagious abortion should be further encouraged, but only on a voluntary basis. The Committee considered also whether or not a system of regular medical inspection of dairy personnel should be introduced, but came to the conclusion that, however desirable this would appear to be, it would mean discrimination between dairy and other workers, and on this account could not be adopted.

In this country, since the beginning of the War, substitutes have had to be found for milk in ice-cream. The Government decided to confine the use of milk to essential purposes in connexion with the proper nutrition of the population, except at times when more than enough was available. Nevertheless, a new policy has been laid down as regards the preparation and selling of ice-cream under hygienic conditions, bacterial standards have been fixed and this supervision will help to eliminate the circumstances which give rise to food poisoning. The bacteriological grading of ice-cream has been fully discussed in two reports (10, 11), and by Wilson (12).

BOVINE TUBERCULOSIS

Although during the War years of 1939–45 there was a marked increase in the number of cases of tuberculosis in Great Britain, and although there has been no decline in notifications since the end of hostilities, it has been noteworthy that this increase has not been attributable to an increased number of infections with the bovine type of bacillus.

(1) *The presence of tuberculous infection in milk and cattle*

In Great Britain, in 1945, a discussion took place at the Royal Society of Medicine (13), in which the various problems connected with the control of milk were reviewed by medical, veterinary and dairying experts. The discussion centred round pasteurization, eradication of bovine disease, suitability of premises on dairy farms and control of personnel engaged in the production of raw milk.

Abel (14), in a discussion on the presence of tubercle bacilli in milk, stated that in 1943 in England and Wales there were 4000 deaths from non-pulmonary tuberculosis, so that some 1200 deaths were presumably due to bovine infection. In the same year there were 14,000 cases of non-pulmonary tuberculosis which meant 4000 due to bovine infection. Half the deaths from non-pulmonary infections occurred in children under 14 years. Wood (15) stated in the same discussion that 40% of all dairy cows slaughtered in this country were found to have tuberculous lesions, and a proportion varying from 2 to 21% of dairy cows, with an average of 7% over the whole country, secreted living tubercle bacilli in their milk. All the bulked milk supply coming into London contained virulent tubercle bacilli, often in large numbers.

Adair (16), in an article in the *Glasgow Herald*, discussed the progress that had been made in the cleaning up of British cattle herds. Whereas of England's total cattle stocks of 6,335,362 only 5·5% were attested animals, 21·3% of 931,164 cattle were attested in Wales, and the Scottish figure was 27·27% out of total stocks of 1,472,013 head. Bledisloe (17), in a letter to *The Times*, commenting on the tardiness of the Government in implementing their declared policy of cleaning up counties as regards tuberculous dairy herds, and to the apathy of a large proportion of our dairy farmers in assisting the process, stated that it was estimated that 40% of the cows are affected in Great Britain, as compared with 0·5% in the United States, 2% in Canada and 4·5% in New Zealand. The incidence of the disease in Switzerland, the Netherlands, Denmark, Belgium and France was said to vary between 20 and 40%. This letter brought a further one from the Agricultural Attaché at the Danish Embassy, that eradication of tuberculosis in Danish dairy herds had made rapid progress, and that in July 1947, 97·4% of herds were free from tuberculous

infection. Ritchie(18) gave some very interesting figures as to the eradication of tuberculosis in Great Britain. At the end of 1947 there were 30,436 herds distributed as follows:

	Attested herds	Attested cattle	Attested cattle (% of total cattle population)
England	9,445	518,412	8·3
Wales	11,099	229,210	25·5
Scotland	9,892	452,397	31·0
Total	30,436	1,200,019	

Further, in Zetland, in Scotland, all herds were attested and only 0·2% of cattle were found to be reactors. In some districts in Wales the percentage of reactors was also very low. Other counties also had a high percentage of dairy cows in attested herds, as:

	Percentage of cattle		Percentage of cattle
Bute	84	Carmarthen	64·6
Ayr	79	Dumfries	61·6
Cardigan	72·5		

As regards foreign countries Indra(19), in the Punjab, examined material from a small series of twenty-one cases of adenitis and found only human tubercle bacilli. In discussing the reports of other workers in India, he stated that the percentage of tuberculous animals in the hill herds and the rural areas is negligible, but the incidence is very high in dairy farms.

(2) *Control and eradication of bovine tuberculosis*

The control and eradication of bovine tuberculosis, with its direct relationship with the human disease, is being actively practised. In Great Britain, Dalling(20) mentions four methods for the control and eradication of tuberculosis in cattle: (*a*) segregation of healthy calves, (*b*) detection and disposal of infected animals, (*c*) creation of immunity or resistance to the disease by vaccination, and (*d*) treatment of tuberculous animals. No matter which method or combination of methods is employed diagnosis is of prime importance, and in cattle depends on the use of Tuberculin. Francis(21) published a book on bovine tuberculosis. The contents included a description of the incidence of tuberculosis in cattle in various countries, the pathogenesis of the disease in relation to route of infection, and the development of lesions in different parts of the body, an account of tuberculosis of bovine origin in man, and finally the detection and control of tuberculous infection in cattle. The sections on the excretion of tubercle bacilli in milk, the relation of bovine to human tuberculosis, and the incidence of bovine-type infection in man, give an up-to-date summary of the literature on these subjects.

In the United States, a report by the Public Health Service(22) states that the aim there is to clear the country of infected cattle as determined by the tuberculin test, and to pay compensation when the reactors are slaughtered.

From Denmark, Holm(23) reports that tuberculosis in cattle has been eradicated from most of the islands, and from north-east Jutland. The result has been that the incidence of infections of man with the bovine organism has fallen, a fact confirmed in the laboratory where each strain is typed.

(3) *The epidemiology of the human infection*

In spite of preventive measures bovine tuberculosis still remains a problem to the public health workers in Great Britain. Bibby(24) accepted the fact that pasteurization in London was responsible for the elimination of much of the bovine tuberculosis under the age of 15 years, but argued that in the age groups over 15 years there was no such reduction. In fact, he suggested that owing to the absence of infection under 15 years there was more risk of acquiring infection after that age, and that the death-rate for cases of non-pulmonary disease over 15 years had declined less rapidly in London than in rural areas of England and Wales. Goodman(25), commenting on the annual report of the Ministry of Health for 1945, states that if it is assumed that 23·3% of non-respiratory

and 1% of the respiratory deaths are attributable to the bovine tubercle bacillus, the deaths from milk-borne tuberculosis were 1147, and although this is an improvement on the corresponding figures for 1920 of 2577, nevertheless, it is a truly disgraceful state of affairs. Lethem(26) states that the incidence of all forms of non-pulmonary tuberculosis is not a satisfactory guide to the incidence of bovine tuberculosis, since about 70% of all such cases are due to the human type of bacillus, whereas most cases of abdominal tuberculosis are due to the bovine type. In London, pasteurization of milk was started in a crude form about 1903: by 1911 about 20%, by 1930 about 90%, and by 1939 about 98% was efficiently pasteurized. On the other hand, much less pasteurization is performed in county boroughs and urban districts, and very considerably less still in rural districts. When a comparison was made between the incidence of abdominal tuberculosis in London and other areas, it was found that by 1944 the death-rate in children under 5 years had fallen in rural districts to about one-quarter, in urban districts to one-ninth, in county boroughs to one-twelfth, and in London to one-twenty-third of the 1921 figure. The author concludes with the statement that in the light of these facts it can hardly be argued that raw milk is as safe as pasteurized milk. Blacklock(27) reports from Scotland that bovine-type infection was responsible for 80·8% of seventy-eight cases of abdominal tuberculosis in children during the period 1914–32, and for 64·7% of seventeen cases in 1943–4; it was also responsible for 70·2% of 131 cases of cervical gland tuberculosis in 1914–35, and for 67·5% of 160 cases in 1943–4. He gives interesting figures, which show that the rural inhabitant is more liable to bovine infection than the urban; thus, of cervical adenitis, 77·2% of cases in the country areas and 46·8% in urban areas were of bovine type; 8% of cases of tuberculous meningitis in large burghs and 15·9% in rural areas were infected with bovine strains; in surgical tuberculosis 20·1% of cases in burghs and cities were caused by bovine infection, and 45·4% in rural areas. He also gives figures to show the comparative incidence of bovine infection in cerebral and surgical tuberculosis in the different areas in Scotland. The western area has 6·1% of cerebral cases, and 27·8% of surgical cases caused by the bovine type, while in other areas the figures range from 8·3 to 27·3% and 24·0 to 54·2%. The author emphasizes the necessity of preventing infections in young children, and states that bovine tuberculosis can be controlled much more easily than can human-type infections. Lloyd(28), commenting on environmental factors in the prevention of tuberculosis, stated that, even at this time, some schools still provided children with raw, untreated milk. Finally, McKinlay(29) has noted that with the onset of war the favourable downward trend in tuberculosis mortality in Scotland was reversed. During 1940 and 1941 the increase was considerable, but thereafter there was a slight improvement in the death-rate for both forms until 1946 and 1947, when an increase in deaths from pulmonary disease again occurred, although the death-rate from other forms tended to decrease. If the pre-war trend had continued, the expected rate in 1947 would have been 450 instead of the actual 659 per million for pulmonary tuberculosis, and 104 instead of 146 for non-pulmonary forms of the disease.

By way of comparison the figures given for the U.S.A. by Halpin(30) are extremely interesting. There the death-rate from all forms of tuberculosis was only 364 per million. Again Meyer(31), in a paper on the eradication of human tuberculosis by epidemiological methods in Minneapolis, states that in the U.S.A. the veterinarians and their allies have so nearly eradicated tuberculosis from cattle that it is rare for human beings to become infected with the bovine type of bacillus. However, as such infections still occur, one must consider the possibility in some cases of the greater likelihood of cattle contracting infection from people than vice versa. The author quotes Tice as reporting the case of a man who had pulmonary tuberculosis caused by the bovine type of bacillus, and who infected ninety-seven of his cattle which had to be slaughtered.

Ruys(32) gave a detailed account of bovine infections in Holland. Thus in 1933–8, in children up to 15 years of age, bovine infections formed 9% of cases of pulmonary tuberculosis and 21% of other forms of tuberculosis in towns with more than 100,000 inhabitants, while for the rest of the country the corresponding figures were 9 and 43%. In 1940, pasteurization was made compulsory for all milk sold in Amsterdam, and as a result the bovine pulmonary infections in children under 15 years during the period 1940–4 fell to 1·8%. In other forms of tuberculosis the incidence of the bovine type fell from 17·2 to 6·8% in those under 15 years and from 12 to 6·8% in the

older age groups. One of the striking features of tuberculosis in Amsterdam under war-time conditions was the marked increase in cervical adenitis, caused by the human type and occurring particularly in people over 50 years of age. Evidently the poor nutrition of the people predisposed them to an increasing number of such infections.

From Denmark, Sigurdsson(33) published his results in a monograph of extensive research on the risk of infection with bovine tuberculosis to a rural population, with special reference to pulmonary tuberculosis, and there are three sections devoted to: (1) an introduction describing the types of tubercle bacilli, their stability and the methods used for their determination; (2) an historical survey dealing with the ways of infection in pulmonary tuberculosis and the distribution of bovine tuberculosis in man in various countries, as compared with the occurrence of tuberculosis among cattle in these countries; and (3) the writer's investigations. The author investigated the type of tuberculous infection in 566 patients with pulmonary tuberculosis, mostly from west and south Jutland and classified them into rural, rural-urban and urban inhabitants. Altogether, 165 patients from rural areas with pulmonary tuberculosis were type-determined, and of these sixty-seven or 40·6% showed the bovine type; while among thirty-nine urban and rural patients eleven or 28·2%, and among the 362 urban patients thirteen or 3·6% had tuberculosis of bovine origin. The author believes that the percentage infected with bovine type bacilli is in direct relation to the contact of the patients with cattle. Serial examination was made of 680 out of a total of 745 individuals on 206 farms in the rural parish of Glostrup. All the inhabitants were examined by X-rays, and children under 15 years were tested with tuberculin, while information was obtained from the veterinary services about the amount of tuberculous infection in the herds. Evidence of scrofulosis was found in 3·0% of persons who had been in contact with tuberculous herds, and only in 1·4% of those in contact with tuberculin negative herds. Further, X-ray changes which might indicate recent or healed pulmonary tuberculosis were demonstrated more frequently in the examinees who had been in contact with tuberculous cattle than in those who had been in contact with tuberculin negative reactors. Leaving out the changes of a more questionable character and reckoning only pulmonary tuberculosis, old tuberculosis of the lungs, or calcified primary complex, the percentages were 4·8 and 0 respectively. From Denmark, also, Knudsen(34) stated that in the 5-year period 1940–4, the bovine type of bacillus was found in six out of sixty-two patients with tuberculous osteomyelitis, thirty out of sixty-five with neck glands, six out of forty-nine urinary tract infections, and thirteen out of 113 cases of pulmonary tuberculosis. It was much more frequent in young persons up to the age of 20, after which the incidence was much reduced. Again, bovine infections were more frequent in those individuals who came from rural areas than among those whose domicile was in a city.

SALMONELLA INFECTIONS

(1) *General*

This group of organisms, which is comprised of some types causing continued fever and others gastro-enteritis in man, continue to be the subject of much investigation. Kauffmann(35) has given a detailed list of 142 strains, 133 of which come within the 'O' groups A to I. Edwards, Bruner & Moran(36) have described the occurrence and distribution of types in man and animals in the United States. A total of 12,331 cultures, derived from 7365 outbreaks of disease and asymptomatic carriers, were identified, and the following table shows those types recovered from ruminants:

Salmonella *organisms found in ruminants*

	O	C		O	C
paratyphi B	7	7	*dublin*	19	20
derby	4	4	*pullorum*	1	1
typhi-murium	39	41	*anatum*	2	3
bredeny	2	2	*give*	1	1
cholerae-suis	15	15	*new brunswick*	1	1
newport	2	2	*worthington*	1	2
kentucky	0	1	*poona*	1	1
enteritidis	5	9	*minnesota*	1	1

O, number of outbreaks; C, number of cultures.

A particular feature of this investigation was the recovery of *S. paratyphi* B from turkeys, chickens, cattle, sheep, swine, dogs, mice, monkeys and egg powder. The authors suggest that domestic animals may act as a reservoir of *S. paratyphi* B and that this finding also adds weight to the suggestion made by Hinshaw, McNeil & Taylor(37) that domestic animals may contract *Salmonella* infections from human cases and carriers. Wolf, Henderson & McCallum(38) also give a detailed account of the various *Salmonella* organisms which they have encountered in domestic animals including cattle, but their investigation did not show any types beyond those already described by Edwards and his colleagues.

Craigie & Felix(39) have reviewed the position in regard to the typing of typhoid bacilli with Vi bacteriophage, which reveals strain differences that cannot be demonstrated by any serological method, and which is extremely useful in epidemiological investigations. They give details of the action of some twenty-four phages, and make the comment that work is being continued on the phage typing of *S. paratyphosus* B and *S. typhi-murium*. Cruickshank(40) discussed the value of phage typing as a means of tracing the sources of infection in Devon, where during the years 1941–4, eighty-three cases had been reported, and where by this means eight different foci of infection could be determined.

(2) *Food poisoning*

All the recent *Salmonella* food poisoning outbreaks due to milk seem to have *S. dublin* as the etiological cause. Thus, Pullinger & Millar(41) described two outbreaks. The first, in Johannesburg in September 1944, involved at least 100 cases, while the second occurred in the same town about a year later and involved 150 individuals. The first outbreak was traced to the milk supply from one particular farm where a cow was found to be ill with mastitis and her milk contained *S. dublin*. In the second outbreak, investigations failed to detect a carrier cow amongst the animals on the various farms which supplied milk to the depot from which the milk was distributed. O'Kelly & Hayes(42) described another outbreak in Ireland, in which 118 people were affected and which was traced to a farm where one particular cow was excreting *S. dublin*. Henderson, Michie, Rae & Smith(43) described an outbreak of food poisoning in the village of Kincardine O'Neil in Aberdeenshire, on 7–9 September 1947, in which ninety-seven people, almost half the total population of the village, were involved. Investigation at once showed that the only common food substance was milk, and when the dairy which supplied it to the village was visited it was found that a cow had been taken ill about 5 September and was so ill on the 6th that the veterinary surgeon was called to see her. The animal died on the 7th and the carcass was removed to the knackery. The herd at the farm consisted of some sixteen cows and several calves, two of which had diarrhoea. From the material obtained post-mortem from the cow, and from the faeces of both calves, *S. dublin* was obtained.

Food poisoning due to cheese, in which the milk from which it was made was contaminated from an outside source with *S. typhi-murium*, has been the subject of a paper by Tucker, Cameron & Henderson(44). Beginning on 26 March 1946, and extending over a period of 1 week, 250 cases of food poisoning occurred amongst the residents of six towns in West Tennessee, and 100 cases in Lawrence County, Illinois. Investigation revealed that the only common food substance was cheese, and when samples were examined bacteriologically *S. typhi-murium* was recovered without difficulty. At the creamery at which the cheese was prepared it was learned that a 10,000-lb. vat was half-filled with milk when a dead mouse was found floating in it. The mouse was fished out and the vat filled. A sample of the infected cheese was held in a refrigerator, and *S. typhi-murium* was actually recovered from it for a period of up to 10 months after it had been manufactured. The authors point out that pasteurization alone would not have prevented an occurrence of this sort.

(3) *Enteric infections*

The number of cases and outbreaks of enteric fever occurring annually become smaller and smaller; the annual mortality from typhoid and paratyphoid fever per million living in England and Wales has decreased from an average of 321 in the period 1871–80 to five in the period

1931–5, and during the War years 1939–45 ranged from 1 to 4. Marmion & Martin (45) describe an outbreak of eleven cases of typhoid fever which occurred in Derbyshire and Leicestershire. All the strains of *S. typhi* isolated, although untypeable by the Vi-phage method, possessed a distinctive character in being resistant to polyvalent anti-salmonella O bacteriophage, which suggested that the patients were infected from a common source. The epidemiological evidence indicated that a strawberry milk drink purchased at a café in the area was the vehicle of infection, but how this occurred was not determined. Wallace & McKenzie (46) reported on a paratyphoid B outbreak which occurred during February and March 1946 in the Wootton district of the Isle of Wight. In all, sixty-two persons were affected, thirty-three being clinical cases and twenty-nine symptomless excreters, and the infecting strain belonged to Vi-phage Type 1. A chain of infection was traced from two carriers in a distant house by way of a cesspool and stream, on to the field of a dairy farm in which the dairy cows grazed. *Salmonella paratyphi* B of Vi-phage Type 1 was isolated from carriers, from the cesspool, from the stream, and from the muddy pasture. Thomson, Stephen, King & Thomson (47) recorded an outbreak which occurred in Glamorgan and which involved fifty-five cases. The milk had been adequately pasteurized, but the water which was used for cleaning the milk utensils was highly contaminated and was actually found to contain *S. paratyphosus* B.

BACILLARY DYSENTERY

A few instances of outbreaks of bacillary dysentery due to milk have been reported in the recent literature. Muir & Vollum (48) reported on an outbreak of Sonne dysentery in which sixty out of eighty-six boarders and sixteen out of ninety-two day pupils were affected at a school. The milk supplied was tuberculin tested, but when the farm was visited one out of the four dairy workers had had the disease and was still carrying the germs. Rae & Smith (49) described in detail an outbreak which occurred in Aberdeen and which involved 252 individuals out of a total of 423 exposed. The cases were distributed equally between the sexes, but the age incidence was slightly higher in those under 10 years. The vehicle of infection was milk from one retail dairy which did not itself produce milk but daily obtained 60 gallons already pasteurized in bulk from the Milk Marketing Board, and which was then bottled at the dairy. Investigation showed that three members of the dairyman's family and one adolescent male worker had had definite symptoms of dysentery, and specimens of faeces from these all showed a profuse growth of *Shigella sonnei*. It is unfortunate that it should be possible to pasteurize milk, sell it in bulk, then retail it, contaminating it in the process of distribution. Feemster (50) stated that in Massachusetts, U.S.A., a small outbreak of thirteen cases due to *Sh. sonnei* occurred in Fall River. The infection was introduced into the dairy by a small daughter. Henderson *et al.* (43) reported on two outbreaks of bacillary dysentery which occurred in the north-east of Scotland and which were due to infected milk. In one outbreak, eighty individuals in a village of some 500 inhabitants were infected with *Sh. sonnei*. In another small town, twenty-one cases were notified and proved to be suffering from an infection with *Sh. manchester*. On inquiry many more cases were found to have occurred but were not notified. In both instances the sequence of events was similar. A child living at each dairy farm was the first to be taken ill, the immediate contacts were the dairy workers, who next became infected and continued with their work in an infective state, and thus contaminated the milk supply. It is interesting to note that in the north-eastern area of Scotland between 1933 and 1945, 454 cases of bacillary dysentery were known to have been caused by *Sh. newcastle*, which gives different biochemical reactions, but has the same serological characteristics as *Sh. manchester*. The organisms had not, however, been recovered from any case during 1946 and 1947, and during the whole period the *manchester* variety had never been encountered. It is presumed, therefore, that the *manchester* type had been imported into the north-east as a result of various troop movements which had occurred.

DIPHTHERIA

Ever since the diphtheria immunization campaign became effective in this country the number of cases and deaths have been very greatly reduced. Many large towns can now record for the first time that not a single fatality has occurred during the past 2 years. On the continent during the

war period the incidence of diphtheria was high and the disease was very severe, but since then there has been a general decline. Another marked feature of the war years was the occurrence of cutaneous diphtheria in Palestine, Iraq, Persia and in the prisoner-of-war camps under Japanese control. The occurrence of the milk-borne form has also suffered a decline. During the past few years the only outbreak is that described by McKenzie (51), in the town of Brighton in the State of Victoria, Australia. In an orphanage thirty-five out of 120 children became bacteriologically positive for *Corynebacterium diphtheriae intermedius*. As the children had been previously immunized only two had tonsillar membrane, but a number of others had mild sore throats. At the dairy which supplied the milk a milker had a diphtheritic ulcer of the nose, apparently the result of a clinical attack some 8 years previously.

STREPTOCOCCAL INFECTIONS

There has been a relatively low and mild incidence of scarlet fever throughout this country during the past number of years, but streptococcal sore throat appears to have had its usual prevalence. On the other hand, the reports in the literature on milk-borne streptococcal infections have also become relatively few in number.

Buch (52) described an epidemic of scarlet fever which occurred in Glostrub in Denmark, and involved 207 individuals. Group A, type 1, haemolytic streptococci were found in the patients, but bacteriological examinations of the staff at the dairy, and of the milk from the various farms which supplied it, failed to show the source. All the cases occurred within 5 days, the greatest number being on the second day of the outbreak, and this led to the conclusion that the milk was affected on 1 day only. This outbreak was estimated to have cost kr. 100,000, and the number of patient-days was 5·969. Evans (53) summarized the findings on twenty-nine milk-borne outbreaks of human streptococcal disease which had been reported. They were all traced to bovine infections, and had been studied to determine the serological type of the causal organism. The evidence that the nature of the outbreak, whether sore throat or scarlet fever, depended upon the type of the *Streptococcus* was fully discussed. She found that outbreaks caused by type 3 were usually classed as scarlet fever; those due to the related types 11, 12 and 27 usually produced, though not always, sore throat, and those due to type 13 caused a sore throat which clinically differed from that usually found in scarlet fever. Mattick (54) and Mattick & Hirsch (55, 56) have given an account of a substance 'Nisin', which they have been able to prepare from lactic streptococci and which was active *in vitro* and *in vivo* against many types of organism including *Str. pyogenes*. Such an observation would, at first sight, seem to have some significance in the control of milk infections due to haemolytic streptococci if lactic streptococci were present.

STAPHYLOCOCCAL INFECTIONS

Staphylococcus aureus is fairly frequently found in milk, but only occasionally is there evidence that it is acting in the role of a pathogen for human beings.

The staphylococcal enterotoxins and haemolysins have usually been regarded as separate and unrelated entities, but reports have appeared which suggest a possible relationship between the beta haemolysin and the enterotoxin. Both these substances are heat stable, and in kittens both give rise to vomiting after intraperitoneal injection, but Surgalla & Hite (57) have examined this problem afresh, and have concluded that the haemolysins and enterotoxins are separable and distinct entities. For the epidemiological investigation of staphylococcal outbreaks, the only successful method of identifying particular strains has been by phage-typing. Wilson & Atkinson (58) have described their method of preparing lytic filtrates with which they were able to identify twenty-one staphylococcal types. Of 1340 strains of staphylococci examined, 60·4% were successfully typed, 22·6% were acted on with phage but were not typed, and 17·0% proved insensitive. The authors found the method very useful in the investigation of outbreaks of food poisoning, and cross-infections. Macdonald (59), on the basis of coagulase production, found *Staph. aureus* in accredited milk as follows:

Examination of milk samples for Staphylococcus aureus

No. of samples examined by		No. containing *Staph. aureus*	Percentage positive	Percentage of positive milks giving on direct plating	
(1) Direct plating	320	102	35	Less than 1000 per ml.	74
(2) Plating after incubation	280	157	56	More than 1000 per ml.	26

By phage typing it was shown that the great majority of milk strains and those isolated from clinical cases of bovine mastitis belonged to one provisional phage type, Type 42D. Staphylococci of the same phage type were isolated in a small number of cases from the hands of milkers. Hussemann & Tanner(60) have investigated the relation of certain cooking procedures to staphylococcal food poisoning, particularly in determining the thermal death time of the organism in cream-filled pastries. The particular strain which was under test was able to withstand heat at 55° C. for 30 min., but was killed in 3 min. at 85° C.

UNDULANT FEVER

In recent years, although a number of interesting papers on various aspects have been published, little new has been added to our knowledge of the bacteriology and epidemiology of this disease. It may be that the problem of the disease in animals will be effectively controlled by eradication and vaccination, or it may be that an effective antibiotic or combination of antibiotics will be found.

In the United States of America, Borts(61) said that the five mid-western states of Iowa, Illinois, Minnesota, Kansas and Wisconsin, reported 10,133 cases of undulant fever during the years 1905 to 1943, and this constituted 25% of those reported for the entire United States. Not all of this disease is due to *Brucella abortus*, since many of the cases are infected with *Br. suis*. The authors suggest that the relative infrequency of human infections with highly infected milk is due to the non-survival of the organism after contact with gastric juices. In South Africa, Lewis, Bersohn & Richardson(62) commented that little is known of the actual incidence of *Brucella* infections in cattle there. They examined 212 composite samples of milk and found that 150, or 70%, showed the presence of whey agglutinins in dilutions of 1/10 or over. A further ninety-two samples were inoculated into guinea-pigs but only three produced *Brucella* infections. Again, Jordan(63) has noted an increase in the incidence of undulant fever in the U.S.A. It rose from 1·99 per 100,000 in the years 1930–9 to 3·11 in 1940–6. The incidence in the different states varied widely, and even reached 18·5 per 100,000 in Vermont. The author has studied a series of 2405 cases as they occurred in Iowa, and in a quarter of these there was no history of contact with farm animals, so it was presumed that the infection in these was due to drinking raw milk. On the other hand, in the bacteriological typing of 434 strains isolated from human cases, 30% were *Br. abortus*, 58% *Br. suis* and 11% *Br. melitensis*. Steele & Hastings(64) gave an account of an epidemic of twenty-eight cases which occurred at Federalsburg, Maryland, and was traced to a heavily infected milk supply. In Spain, Villalonga(65) has found that in fifty-two provinces in the years 1940–4 there had been respectively 6376, 6419, 5404, 4182 and 4290, making a total of 26,671, with an average mortality rate of 1·8%. For England and Wales, Dalrymple-Champneys(66) has contributed an extensive and complete study on the epidemiological aspects of undulant fever based on a study of nearly 900 cases over a period of 20 years. In 871 cases in the series investigated, all, so far as was known, were due to *Br. abortus*, and the duration of the fever varied from 1 to 47 weeks with an average of 15·3 weeks and a mortality rate of 2·3%. Males were affected more frequently than females, there being 540 males and 329 females, or, excluding those persons having had possible contact with infected animals, their numbers were 497 males and 318 females, and, as the author points out, this shows that the sex difference, found in every county where the disease occurs, is not occupational in origin. As regards age, only eight children under 5 years were affected and thirty-six under 10 years, while the highest incidence was in the decennium 30–40 years. The evidence that infection came from milk is summarized as follows:

Evidence of infection from milk

Drank raw milk or cream	Contagious abortion reported present	Milk whey agglutinated *Brucella*	*Brucella* grown from milk	Cows' blood serum agglutinated *Brucella*	*Brucella* grown from cows' blood
634	167	36	29	17	4

London, where well over 90% of the milk supply is pasteurized, provided only thirty-three cases, and in fourteen of them it had been ascertained that raw milk had been consumed. The author discusses the legal aspects of this infection and states that the Ministry of Health have taken the view, in agreement with the Ministry of Agriculture, that when a cow is secreting the organism of *Br. abortus* in her milk, it is conclusive evidence that she is suffering from an infection of the udder which is likely to convey disease, and therefore this comes within the terms of Section 25 and Part 1 of the First Schedule of the Food and Drugs Act, 1938, and the Regulations or Orders thereunder. In the Argentine, the issue of the *Review of the Medical Association* for March 1948, contains a series of seven papers (67–73) describing the various aspects of brucellosis in that country. These papers dealt with the chief bacteriological characteristics of the brucella group of organisms, the epidemiology of human brucellosis in the Argentine Republic, the clinical and radiological aspects of the disease and, finally, diagnosis and treatment. The epidemiological paper deals with a study of 2000 cases, 438 of which were examined bacteriologically. In certain provinces the disease was due to the *melitensis* variety, but in other areas was largely due to *suis* and *abortus*.

BOVINE LEPTOSPIROSIS

The first description of bovine leptospirosis was given by Michin & Azinow (74) in 1935 and pertained to an outbreak of this disease in the North Caucasus, but later a more detailed account was given by Semskow (75). The occurrence of a similar disease was reported from Australia by Johnson (76), and from the United States of America by Jungherr (77), Marsh (78), and Baker & Little (79). A series of reports from Palestinian workers on the infection of cattle, goats and sheep, by *Leptospira bovis* and of the possible transmission of the disease to man through the agency of milk appeared in 1948. Thus Bernkopf, Olitzki & Stuezynski (80) reported that the disease in cattle is fairly widespread, and that the economic loss as a result of this infection was considerable. The disease was transmitted artificially to calves and further, sera taken from nineteen human individuals, who had suffered from jaundice and nephritis, gave very high agglutination titres against this organism. All the affected individuals were in an occupational group having close contact with cattle. The same workers (Bernkopf *et al.* (81)) pointed out that one of the earliest symptoms in a cow is the appearance of blood and blood clots in milk, and that blood stream infection is likely to give rise to infected milk. Leptospira were added to certain milk samples which were retained in the incubator for 3 days and then inoculated into calves. The calves responded with a typical infection and the Leptospira could be recovered from them. More recently, Bernkopf & Little (82) have shown that the strains of Leptospira recovered from cows in Palestine and New Jersey, U.S.A., are identical. To what extent human beings can be infected through milk is, therefore, not yet clear, but no doubt further investigations will settle this problem.

Q FEVER

It has been shown recently that Q fever may be to some extent a milk-borne disease. The story of the study of its etiology is that it had two beginnings, one in Australia, the other in the United States. In each instance the infectious agent was classified as a rickettsia and these were soon shown to be identical. Dyer (83) states that although the etiological agent of Q fever was classified as a rickettsia, it was recognized early that certain differences exist between it and all the other rickettsiae. Morphologically, tinctorially and culturally, it is similar to other rickettsiae, but on the other hand, it does not develop agglutinins for any known strain of *Bacillus proteus*, and it readily passes bacterial filters. These variations, coupled with the fact that the organism shows

greater resistance to chemical and physical agents, justify the setting up of the Q fever organism as the type organism of a new genus *Coxiella* (Philip (84)). Hence *C. burnetti* supersedes *Rickettsia burnetti* and *R. diaporica*. In Australia, Derrick (85) first described it as an abattoir disease; then it was found to occur amongst the personnel on dairy farms, and although many additional cases have been reported from Australia no very definite epidemiological deductions have been drawn. The clinical characteristics of the human disease is that of an atypical pneumonia with a low mortality rate. Burnett & Freeman (86) found that the etiological agent passed through bacterial filters, could be demonstrated in the spleens of infected mice, and could be cultured on the chorio-allantoic membranes of a developing chick embryo, while Derrick & Smith (87) succeeded in transferring the infectious agent to guinea-pigs and other animals. An outbreak resembling influenza, which occurred in Athens in 1943, has been described by Caminopetros (88), and was known as 'Balkan Grippe'. It occurred amongst German troops in the Balkans (Dennig (89)), and amongst the civilian population of Bulgaria (Imhauser (90)) and Roumania (Combiesco, Vasiliu & Dimitresco (91)), Robbins & Ragan (92); Robbins, Gauld & Warner (93) and Robbins, Rustigian, Snyder & Smadel (94) recognized the disease among American troops in Italy, and it occurred amongst men who had been repatriated to the U.S.A. (Feinstein, Yesner & Marks (95); Finland & Lesses (96)).

In the United States outbreaks occurred in stockyard workers in Texas (Cox, Tesar & Irons (97)) and Chicago (Shepard (98)). Then, in 1947, an outbreak occurred in Los Angeles amongst individuals who, though not actually dairy workers, had had some contact with cows. In connexion with this outbreak Huebner, Jellison, Beck & Parker (99) described the recovery of *Coxiella burnetti* from pooled raw milk of four dairies, and in a further report Beck, Bell, Shaw & Huebner (100), in a study of 300 cases in Los Angeles, stated that 32% of those affected used raw milk. Huebner, Jellison, Beck & Wilcox (101) also showed that pasteurization by the H.T.S.T. and the vat-holding method were both effective in destroying the organism. In Greece, the organism has also been recovered from the milk of sheep and goats (Caminopetros (88)). Although the disease may not in all instances be conveyed by milk, sufficient work has been done to establish the fact that it can be excreted in the milk from cows, goats and sheep, and in this way is a potential source of danger to susceptible humans.

VIRUS DISEASES

During the war years outbreaks of infective hepatitis amongst military personnel and the civilian population stimulated further research on the etiology, method of spread, and prevention of this disease. It was shown that the virus was excreted in the faeces and urine. In consequence, it was a disease of communal living and feeding and it flourished where amenities for the maintenance of personal hygiene, ablution, laundry and cleaning services were restricted. Murphy, Petrie & Work (102) described a small outbreak of ten cases which occurred in Forsyth, Georgia, U.S.A., during the period 21 May to 28 June 1945. It involved eight households, six of which had a single case. The evidence gathered during the investigation, while purely circumstantial in character, pointed quite definitely towards a single milk supply as the source of the outbreak, as it was established that, prior to the outbreak, at least two cases of infective hepatitis lived in close proximity. The dairy under suspicion supplied raw milk to twenty-six households with 114 consumers, the figure in each instance being slightly less than 5% of the total in the town of Forsyth, and within the incubation period cases appeared amongst the consumers of this particular supply but not among the non-consumers. The authors concluded that, since contamination of the milk at the dairy was highly probable and since the observed distribution of cases coincided so closely with the distribution of the milk, the possibility of the outbreak being unrelated to the milk supply appeared remote.

In 1947, Great Britain was affected by a very large and widespread outbreak of anterior poliomyelitis, and attention was again focused on probable methods of spread. It is not possible to say to what extent the apparent, the latent, and the carrier case were each responsible for the spread of infection. There can, however, be no doubt that in those affected, the virus may be obtained from either the pharyngeal or faecal secretions or both, and that although pharyngeal secretion may be the source of most contact infections, the faeces may be the chief source of virus in

non-contact infections. Thus Wickman (103), Dingman (104), Aycock (105) have all reported on epidemics which might have been spread by milk. More recently, Goldstein, Harmon & Viets (106) reported on an outbreak of poliomyelitis in a naval training school in which eighteen marked and probably 100 milder cases occurred within an 8-day period. The data accumulated during the epidemiological investigation pointed to food, probably milk contaminated by flies, as the common source of infection. The milk used was mostly raw, frequently had high bacterial counts, and regularly contained flies which had access to human and domestic animal faeces. Macnamara (107, 108), in Australia, said that there were many reasons why raw milk, unscalded cream, and ice-cream, should be suspected as a vehicle of infection. For instance, the increase in type A epidemics and the higher urban increase have coincided with the development of the dairy industry. Further, the lowered incidence of the disease in babies was probably due to more care being taken in using dried or heat-treated milk for their feeding. Lacey (109), in an exhaustive article on the natural history of poliomyelitis, stated that, in a few epidemics, infection had most probably been spread by milk. Macnamara (107) recalls the work by Park (110), in which he showed that the virus of poliomyelitis can survive in milk stored in a refrigerator for 6 months. Lawson & Melnick (111) showed that milk exerts a protective action on the destruction by heat of rodent-adapted and spontaneous mouse poliomyelitis viruses. When infected central nervous system tissue was heated in this medium these strains were able to withstand 5–10° C. more heat for 30 min. than when the infected tissues were suspended in water. In fact, when concentrated suspensions of infected nervous tissue were heated for 30 min. occasional traces of virus could be detected even when the temperature was kept at 65 and 70° C. The authors conclude with the statement that our knowledge of the survival of poliomyelitis virus in milk and ice-cream was inadequate. Although the actual virus has never been recovered from the cow it does seem possible for milk to be contaminated directly from human sources or indirectly by flies.

J. SMITH

THE REGIONAL LABORATORY
CITY HOSPITAL
ABERDEEN

REFERENCES

(1) EDITORIAL (1945). *Lancet*, **1**, 502.
(2) WILSON (1943). *The Pasteurization of Milk*, p. 56. London: Arnold.
(3) EDITORIAL (1945). *Brit. med. J.* **2**, 160.
(4) GREAT BRITAIN: PARLIAMENT. *Measures to Improve the Quality of the Nation's Milk Supply* (1943). Cmd 6454. London: H.M.S.O.
(5) EDITORIAL (1946). *Brit. med. J.* **1**, 655.
(6) CHALMERS (1946). *Hlth Bull.* **5**, 3.
(7) SCOTLAND: DEP. OF AGRICULTURE. *Report of an Investigation into the Economics of Milk Production in Scotland*, 1948, no. 1. Edinburgh: H.M.S.O.
(8) MINISTRY OF FOOD. *Report of the Committee (English) on Milk Distribution*, 1948, p. 24. London: H.M.S.O.
(9) SCOTLAND: DEP. OF AGRICULTURE AND DEP. OF HEALTH. *Report of the Committee on Milk Services, Scotland*, 1949, p. 30. H.M.S.O.
(10) MINISTRY OF HEALTH. Public Health Laboratory Service (1947). *Mon. Bull. Minist. Hlth Lab. Serv.* **6**, 60.
(11) MINISTRY OF HEALTH. Public Health Laboratory Service (1948). *Mon. Bull. Minist. Hlth Lab. Serv.* **7**, 84.
(12) WILSON (1948). *Med. Offr*, **79**, 261.
(13) MATTHEWS (1945). *Proc. R. Soc. Med.* **38**, 253.
(14) ABEL (1945). *Brit. med. J.* **2**, Suppl. p. 34.
(15) WOOD (1945). *Brit. med. J.* **2**, Suppl. p. 33.
(16) ADAIR (1947). *Glasgow Herald*, 18 February.
(17) BLEDISLOE (1948). *The Times*, 30 October.
(18) RITCHIE (1948). *J. Roy. Sanit. Inst.* **68**, 503.

(19) Indra (1946). *Indian med. Gaz.* **81**, 67.
(20) Dalling (1946). *Proc. R. Soc. Med.* **39**, 213.
(21) Francis (1947). *Bovine Tuberculosis.* London: Staples Press.
(22) Editorial (1946). *Publ. Hlth Rep., Wash.*, **61**, 1315.
(23) Holm (1946). *Publ. Hlth Rep., Wash.*, **61**, 1426.
(24) Bibby (1945). *Med. Offr*, **74**, 189.
(25) Goodman (1946). *Bull. Hyg., Lond.*, **21**, 207.
(26) Lethem (1946). *Mon. Bull. Minist. Hlth Lab. Serv.* **5**, 80.
(27) Blacklock (1947). *Brit. med. J.* **1**, 707.
(28) Lloyd (1948). *Brit. med. J.* **2**, 870.
(29) McKinlay (1948). *Hlth Bull.* **6**, 66.
(30) Halpin (1948). *Publ. Hlth Rep., Wash.*, **63**, 1029.
(31) Meyer (1948). *Amer. J. publ. Hlth*, **38**, 516.
(32) Ruys (1946). *Mon. Bull. Minist. Hlth Lab. Serv.* **5**, 67.
(33) Sigurdsson (1945). *Studies on the Risk of Infection with Bovine Tuberculosis.* Oxford University Press.
(34) Knudsen (1947). *Ugeskr. Læg.* **109**, 93.
(35) Kauffmann (1947). *Acta path. microbiol. scand.* **24**, 242.
(36) Edwards, Bruner & Moran (1949). *J. infect. Dis.* **83**, 220.
(37) Hinshaw, McNeil & Taylor (1944). *Amer. J. Hyg.* **40**, 264.
(38) Wolf, Henderson & McCallum (1948). *Amer. J. publ. Hlth*, **38**, 403.
(39) Craigie & Felix (1947). *Lancet*, **1**, 823.
(40) Cruickshank (1947). *Mon. Bull. Minist. Hlth Lab. Serv.* **6**, 88.
(41) Pullinger & Millar (1946). *S. Afr. med. J.* **19**, 313.
(42) O'Kelly & Hayes (1946). *Irish J. med. Sci.* **6**, 615.
(43) Henderson, Michie, Rae & Smith (1948). *Hlth Bull. Dep. Hlth, Scotland*, **6**, 28.
(44) Tucker, Cameron & Henderson (1946). *J. Amer. med Ass.* **131**, 1119.
(45) Marmion & Martin (1946). *Mon. Bull. Minist. Hlth Lab. Serv.* **5**, 205.
(46) Wallace & McKenzie (1947). *Mon. Bull. Minist. Hlth Lab. Serv.* **6**, 32.
(47) Thomson, Stephen, King & Thomson (1948). *Lancet*, **255**, 270.
(48) Muir & Vollum (1945). *Mon. Bull. Minist. Hlth Lab. Serv.* **4**, 220.
(49) Rae & Smith (1945). *Hlth Bull. Dep. Hlth, Scotland*, **4**, 30.
(50) Feemster (1946). *Amer. J. publ. Hlth*, **36**, 777.
(51) McKenzie (1947). *Hlth Bull., Melbourne*, p. 2395.
(52) Buch (1946). *Ugeskr. Læg.* **108**, 199.
(53) Evans (1946). *J. infect. Dis.* **78**, 18.
(54) Mattick (1946). *Endeavour*, **5**, 24.
(55) Mattick & Hirsch (1946). *Lancet*, **250**, 417.
(56) Mattick & Hirsch (1947). *Lancet*, **253**, 5.
(57) Surgalla & Hite (1945). *J. infect. Dis.* **76**, 78.
(58) Wilson & Atkinson (1945). *Lancet*, **248**, 647.
(59) Macdonald (1946). *Mon. Bull. Minist. Hlth Lab. Serv.* **5**, 230.
(60) Hussemann & Tanner (1947). *Amer. J. publ. Hlth*, **37**, 1407.
(61) Borts (1945). *J. Kans. med. Soc.* **46**, 399.
(62) Lewis, Bersohn & Richardson (1948). *S. Afr. med. J.* **22**, 763.
(63) Jordan (1949). *Industr. Med.* **17**, 176.
(64) Steele & Hastings (1948). *Publ. Hlth Rep., Wash.*, **63**, 144.
(65) Villalonga (1945). *Rev. Sanid. Hig. publ., Madrid*, **19**, 509.
(66) Dalrymple-Champneys (1948). *Publ. Hlth*, **59**, 239.
(67) Tey (1948). *Rev. Asoc. méd. argent.* **62**, 109.
(68) Molineli, Ithurrat & Ithuralde (1948). *Rev. Asoc. méd. argent.* **62**, 111.
(69) Basso & Miyara (1948). *Rev. Asoc. méd. argent.* **62**, 118.
(70) De Villafane (1948). *Rev. Asoc. méd. argent.* **62**, 125.
(71) Amuelastegui (1948). *Rev. Asoc. méd. argent.* **62**, 137.
(72) Di Rienzo (1948). *Rev. Asoc. méd. argent.* **62**, 153.

(73) Alberto Videla (1948). *Rev. Asoc. méd. argent.* **62**, 164.
(74) Michin & Aznow (1935). *Sovyet Vet.* **10**, 23.
(75) Semskow (1941). *Z. Veterinärk.* **53**, 7.
(76) Johnson (1943). *Brit. med. J.* **2**, 659.
(77) Jungherr (1944). *J. Amer. vet. med. Ass.* **105**, 276.
(78) Marsh (1945). *J. Amer. vet. med. Ass.* **107**, 119.
(79) Baker & Little (1948). *J. exp. Med.* **88**, 295.
(80) Bernkopf, Olitzki & Stuezynski (1947). *J. infect. Dis.* **80**, 53.
(81) Bernkopf, Olitzki & Stuezynski (1948). *J. infect. Dis.* **83**, 232.
(82) Bernkopf & Little (1948). *Proc. Soc. exp. Biol., N.Y.*, **69**, 503.
(83) Dyer (1948). *Amer. J. publ. Hlth*, **39**, 471.
(84) Philip (1948). *Publ. Hlth Rep., Wash.*, **63**, 58.
(85) Derrick (1937). *Med. J. Aust.* **2**, 281.
(86) Burnett & Freeman (1938). *Med. J. Aust.* **1**, 11.
(87) Derrick & Smith (1940). *Aust. J. exp. Biol. med. Sci.* **18**, 1.
(88) Caminopetros (1948). *Abstr. 4th Int. Congr. Trop. Med. Wash. D.C.* pp. 33–48.
(89) Dennig (1947). *Dtsch. med. Wschr.* **72**, 369.
(90) Imhauser (1943). *Z. klin. Med.* **142**, 486.
(91) Combiesco, Vasiliu & Dimitresco (1947). *C.R. Soc. Biol., Paris*, **141**, 716.
(92) Robbins & Ragan (1946). *Amer. J. Hyg.* **44**, 6.
(93) Robbins, Gauld & Warner (1946). *Amer. J. Hyg.* **44**, 23.
(94) Robbins, Rustigian, Snyder & Smadel (1946). *Amer. J. Hyg.* **44**, 51.
(95) Feinstein, Yesner & Marks (1946). *Amer. J. Hyg.* **44**, 72.
(96) Finland & Lesses (1947). *New Engl. J. Med.* **237**, 255.
(97) Cox, Tesar & Irons (1946). *J. Amer. med Ass.* **133**, 820.
(98) Shepard (1947). *Amer. J. Hyg.* **46**, 185.
(99) Huebner, Jellison, Beck & Parker (1948). *Publ. Hlth Rep., Wash.*, **63**, 214.
(100) Beck, Bell, Shaw & Huebner (1949). *Publ. Hlth Rep., Wash.*, **64**, 41.
(101) Huebner, Jellison, Beck & Wilcox (1949). *Publ. Hlth Rep., Wash.*, **64**, 499.
(102) Murphy, Petrie & Work (1946). *Amer. J. publ. Hlth*, **36**, 169.
(103) Wickman (1907). *J. nerv. ment. Dis.* Monograph series, no. 16.
(104) Dingman (1916). *N.Y. St. J. Med.* **16**, 589.
(105) Aycock (1937). *Amer. J. Hyg.* **7**, 791.
(106) Goldstein, Harmon & Viets (1946). *J. Amer. med. Ass.* **131**, 569.
(107) Macnamara (1947). *Med. J. Aust.* **2**, 717.
(108) Macnamara (1948). *Med. J. Aust.* **1**, 183.
(109) Lacey (1949). *Lancet*, **256**, 849.
(110) Park (1932). *Poliomyelitis*, p. 459. New York.
(111) Lawson & Melnick (1947). *J. infect. Dis.* **80**, 201.

(*MS. received for publication* 15 *August* 1949)